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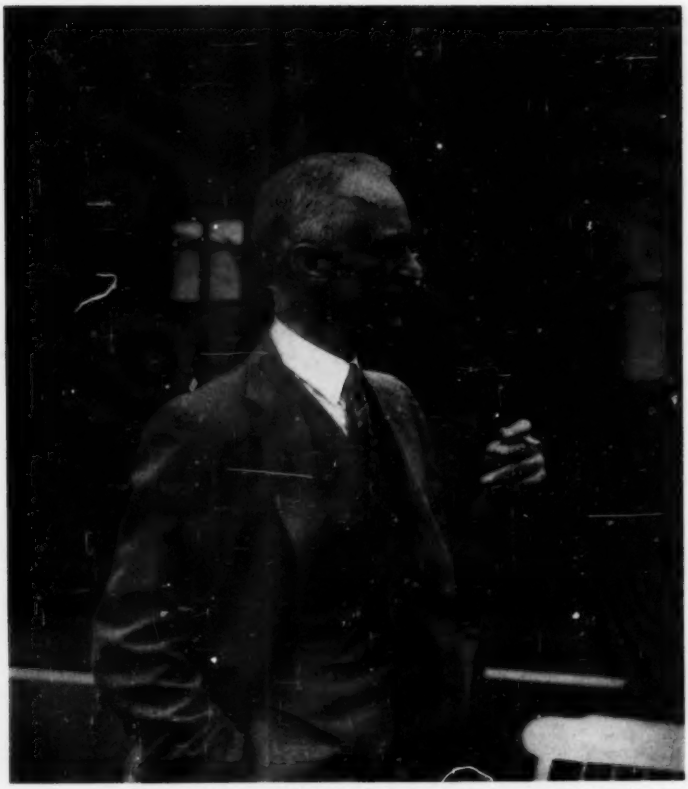

Photograph taken in 1926 by Dr. L. J. Herrmann at the Peter Bent Brigham Hospital

HARVEY CUSHING

UNIV. OF MICH.

Medical
Farrow

Harvey Cushing

1869–1939

A surgeon, artistic in technique; a writer, excelling in the use of the English language; an inspiring teacher, who developed many neurosurgeons to spread far and wide the methods perfected by a master; an experimenter and investigator in the realm of endocrinologic science; an historian, versed in the lore of the past; a neurologist, skilled in the diagnosis of disturbances in brain and nerves; a practitioner, beloved by his many patients; Harvey Cushing has died at New Haven of coronary occlusion on the seventh day of October, 1939, in his seventy-first year.

Trained in succession at Yale (A.B., 1891), at Harvard (A.M. and M.D., 1895) and at the Massachusetts General Hospital (House Pupil, 1895–96) in turn he served the Johns Hopkins University and Hospital (1896–1912), the Peter Bent Brigham Hospital and Harvard University (1912–1932) and Yale University (1933–1939).

In Baltimore Dr. Cushing came under the influence of William S. Halsted, from whom he learned the importance of the experimental method and of detailed records of all observations in clinic and laboratory and that gentleness in the handling of tissues with a minimal loss of blood was far more significant in the success of surgery than speed or even manual dexterity. This training in general surgery prepared him in the best possible way to become the brilliant, pioneering father of neurosurgery and a stimulating, inspiring teacher; before him in the field of brain surgery little success had followed operation; after him many pupils were able, at home and abroad, speedily to extend his leadership and masterfully to make neurosurgery safe in the hands of many.

In Baltimore, too, began the friendship for William Osler that was an important stimulus in the clinical and literary development of Harvey Cushing, the latter to culminate in the splendid Life of Sir William Osler that was published at the Clarendon Press in 1925.

By 1910 a brilliant mind and indefatigable labor in the wards and

O.K.

operating room of the Johns Hopkins Hospital and in the laboratories of the Johns Hopkins Medical School had made of Harvey Cushing a recognized leader in surgery and in its teaching. Little wonder then that in this year (1910), when seeking the man to develop surgery in a new hospital in Boston, all thought naturally centered on him as the ideal man to develop a new Surgery to meet the needs of a Medicine rapidly changing to fit new aspects of science and medical investigation and practice; the call to him to become Surgeon-in-Chief of the Peter Bent Brigham Hospital, then under construction, and Professor of Surgery in Harvard University was accepted; in 1912 he came to Boston, was made Moseley Professor of Surgery and commenced the work that proved so successful, and here he remained until 1932, when he retired at the age of 63, the retirement age set by himself and his colleague on the medical service on the opening of the Peter Bent Brigham Hospital, then to become Surgeon-in-Chief, Emeritus and Moseley Professor of Surgery, Emeritus.

In the twenty year period in Boston he accomplished his major work. Neurosurgery progressively was improved, and men came from far and near to learn this new surgical technique and the ways accurately to make a diagnosis of the neurolesion that needed surgical treatment. Equally, from all parts of the world, came patients to be ministered unto, since there were then scarce any who could perform operations on the brain with safety and success, a condition that persisted only until his pupils returned to their homes to put into practice what Dr. Cushing had taught them. From his pen came important publications; from his laboratory came significant advances in the pathology and physiology of brain, neighboring endocrine structures and brain tumors; books were published; remarkably detailed clinical records were accumulated; students, undergraduate and graduate, were educated; a resident staff was taught the meaning of a meticulously careful, accurate, safe, surgical technique; colleagues on the Hospital Staff and Medical Faculty received inspiring and delightful stimulation; these were years of great productivity and of great influence on the science and practice of surgery.

Then came the interruption of the Great War and overseas service in 1915 and in 1917 to 1919 with a different opportunity for leadership and learning, an opportunity, which took heavy toll on the health

and strength of Dr. Cushing, but which, as always, left a great influence on associates and on surgery itself, especially neurosurgery and neurological diagnosis. Copious diary notes, too, in this period later were converted into pleasing and instructive literature.

Retirement from hospital and academic duties in Boston in 1932 meant no let-up in the activity and productivity of this man, although already his health was meeting the vicissitudes of peripheral vascular disease and gastric ulcer. The last day of his service at the Peter Bent Brigham Hospital ended Dr. Cushing's work as an operating surgeon, and the scalpel was laid aside; now he turned to his laboratory, his accumulated clinical records and his library for another period of activity that continued up to a few days before his death. After a year's work in the laboratory of the Peter Bent Brigham Hospital Dr. Cushing went to New Haven as Sterling Professor of Neurology in Yale University, which position he held from 1933 to 1937, when he became Emeritus Professor and Director of Studies in the History of Medicine, continuing at Yale the activities that he began in Boston in the autumn of 1932.

Honors deservedly were showered upon Dr. Cushing in recognition of his outstanding merit in many fields as have been received by few, if any, other surgeons. The following honorary degrees were given him; *A.M.*, Yale, '13; *LL.D.*, Western Reserve, '19, Cambridge, '20, Edinburgh, '27, Glasgow, '27, Syracuse, '34; *Sc.D.*, Washington Univ., '15, Yale, '19, Harvard, '31, Rochester, '31, Northwestern, '32, Leeds, '35, Oxford, '38; *M.D., hon. causa*, Belfast, '19, Lwow, '26, Strasbourg, '29, Brussels, '30, Budapest, '31, Bern, '31, Amsterdam, '32, Paris, '33; *Lit. D.*, Jefferson, '26, Dartmouth, '29; *M.Ch.*, Trinity College, Dublin, '27; *Hon. F.R.C.S.*, England, '13, Ireland, '18 and Edinburgh, '27. He was made foreign member of Royal Med. Soc. of Budapest, '09, Soc. de Neurologie, Paris, '14, Soc. Medicorum Svecana, '17, Soc. Royale des Sc. Medicales et Naturelles, Bruxelles, '19, Soc. Nat. de Chirurgie, Paris, '19, Soc. de Biologie, Paris, '20, Med. Soc. of London, '21 (Orator, '27), Gesellschaft der Aerzte in Wien, '21, Medico-Chirurgical Soc., Edinburgh, '22, Académie de Médecine, Paris, '23, Assoc. of Surgeons of Great Britain and Ireland, '23, Soc. Medico-Chirurgica di Bologna, '24, Royal Soc. of Medicine, London, '27, Royal Acad. of Medicine in Ireland, '27, Soc. of British Neurological

Surgeons, '27, Soc. Medica Havniensis, '28, Soc. de Neurologie de Varsovie, '29, Verein für Psychiatrie and Neurologie, Vienna, '29, Gesellschaft deutscher Nervenärzte, '29, Soc. Regia Medicorum, Budapest, '29, Acad. Royale de Médécine de Belgique, '30, Royal Medico-Psychological Assoc., Great Britain, '30, Soc. Piemontese di Chirurgia, '31, Kaiserliche deutsche Akademie der Naturforscher, '32, British Med. Assoc., '32, Soc. Nacional de Cirurgia de la Habana, '32, Soc. Radio-Neuro-Chirurgica Italiana, '32, Royal Soc. of Sciences, Upsala, '32, Soc. Italiana di Neurologia, '32, Gesellschaft deutscher Neurologen und Psychiater, '32, Hungarian Ophthalmological Soc., '33, Royal Soc. of London, '33, R. Acad. delle Scienze dell' Istituto di Bologna, '33, North Caucasian Assoc. of Neurologists and Psychiatrists, '33, Norske Medicinske Selskab, '33, Soc. Lombarda di Chirurgia, '33, Soc. Medica Norvegica, '33, Soc. de Chirurgia de Lyon, '34, Royal Acad. of Sciences, Sweden, '34, Indian Acad. of Sciences, Bangalore, '35, Royal Acad. of Sciences, Amsterdam, '35, Österreich. Gesellschaft für Erforschung der Krebskrankh., Vienna, '35, Acad. Nacional de Medicina, Buenos Aires, '35, Soc. de Biologie, Paris, '36, Royal Med. Soc., Edinburgh, '36, Acad. de Chirurgie, '36, Gesellschaft der Chirurgen in Wien, '36, Wien. Biologische Gesellschaft, '37, Harveian Soc. of London, '38, Polish Acad. of Science, '38. He was Charles Mickle Fellow, Univ. of Toronto in '22 and Macewen lecturer, Univ. of Glasgow in '27, received the Cameron Prize from the Univ. of Edinburgh in '24, the Pulitzer Prize in Letters, Columbia Univ. in '26, the Lister Prize Medal, Royal College of Surgeons, England in '29, the Montclair Yale Bowl in '30 and the Henry J. Bigelow Medal of the Boston Surgical Society in 1933, Gold Medal, Nat. Institute of Social Sciences in '35. He was made a member of the Am. Acad. of Arts and Sciences in '14, the Nat. Acad. of Science in '17 and of the Am. Philosophical Soc. in '30. In 1922 he was made Hon. Perpetual Student, St. Bartholomew's Hospital, London. He was a member of the Am. Assoc. of Pathologists and Bacteriologists, Soc. of Neurological Surgeons (Pres. 1920–21), Am. Physiological Soc., Am. Soc. of Clin. Surgery (Pres. 1921), Am. College of Surgeons (Pres. 1922), Am. Neurolog. Assoc. (Pres. 1923), Am. Surgical Assoc. (Pres. 1927). He was Surgeon, Harvard Univ. Am. Ambulance Hosp., Apr.–June '15 and Director, U. S. Base Hosp. No. 5 attached to B.E.F.

in France 1917–19; Senior consultant in neurological surgery, A.E.F., '18; colonel, M.C., U.S.A. He received the Distinguished Service Medal, U. S. A. and was made Companion of the Bath and Officier Legion d'Honneur following his service over seas.

On June 10, 1902, Dr. Cushing married Katherine Stone Crowell of Cleveland, who survives him as do three daughters and one son.

Dr. Cushing's scientific work ranged from studies during his earlier years on bacteriological and general surgical topics to a long series of neurosurgical papers and books that came steadily from his laboratory and clinic from the first three, "Haematomyelia from gunshot wound of the spine" published in 1898 and "Observations upon the neural anatomy of the inguinal region" and "A method of total extirpation of the Gasserian ganglion for trigeminal neuralgia" published in 1900, to the last in 1938, his book "Meningiomas," generally regarded as his greatest clinical monograph. His publications totalled thirteen books and monographs and over three hundred addresses, papers and reports. The titles of his books and monographs, "Pituitary Body," "Tumors of Nervus Acusticus," "Story of Base Hospital No. 5," "Life of Sir William Osler," "Classification of Gliomas," "Studies in Intracranial Physiology," "Pathological Findings in Acromegaly," "Tumors Arising from Blood Vessels," "Consecratio Medici," "Intracranial Tumors," "Pituitary Body and Hypothalamus," "From a Surgeon's Journal" and "Meningiomas" epitomize the clinical, scientific and literary activity of this truly remarkable man. To him must be credited, too, the training and inspiring of a very large number of pupils, whose productivity, and it has been very great, in a sense must be attributed to Dr. Cushing.

For twenty years I, the author of this tribute, had an intimate association with Dr. Cushing at the Peter Bent Brigham while we served as chief of the surgical and medical services, respectively. They were happy, profitable years of coordinated effort to perfect the work of the hospital and the teaching of medical students and resident staff, efforts to whose success Dr. Cushing contributed very greatly. Discussion of means and method were free and active; from these developed a friendship and affection that was sincere and enduring, ever without rancour, even when there were differences of opinion, and discussions always ended with mutually accepted ways of coordinating

effort. Memory today brings many happy recollections of that period of mutual activity to a common end to which Dr. Cushing contributed so richly and so productively throughout life.

HENRY A. CHRISTIAN.

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METABOLIC STUDIES AND THERAPY IN A CASE OF NEPHROCALCINOSIS WITH RICKETS AND DWARFISM

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The patient, upon whom the studies here reported were performed, had a condition which was, it is believed, renal in origin, and which resulted in rickets, but which was not "renal rickets." This latter condition, as pointed out in a previous publication (1), is not rickets but, rather, osteitis fibrosa. It should be emphasized, furthermore, that the various abnormalities in the patient here reported are not disconnected phenomena, but probably constitute an integral syndrome. An important piece of evidence for this statement is the fact that a patient with almost exactly similar findings, whose case history will be abstracted below, was seen by one of us (F. A.) and has been reported briefly by Butler, Wilson and Farber (2). For the condition the following descriptive name is suggested, "Nephrocalcinosis with rickets and dwarfism."

CASE HISTORY

Mildred J. (#9934), aged 13 years, was sent to the Massachusetts General Hospital on February 13, 1937, by Dr. Charles Bradley from the Emma Pendleton Bradley Home in East Providence, Rhode Island, in order that studies might be performed to determine whether or not hyperparathyroidism was present. Her chief complaints were dwarfism and inability to walk.

She weighed 9¼ pounds at birth. Dentition was delayed until between the ages of 18 and 24 months. Otherwise she was considered essentially normal until a severe attack of measles at 4. From that time on she grew very slowly and had trouble with her legs, the left becoming bowed and the right knock-kneed. At the age of 5 she fell against a doll carriage and fractured the right femur two inches above the knee. The fracture healed without complications.

At 11, because of persistent deformities, osteotomies were performed on the right femur and the left tibia at the Memorial Hospital, Pawtucket, Rhode Island. Three months later she fractured the right femur at the site of the osteotomy. In July 1936, she passed the first of several urinary calculi. In August 1936, while playing, she fell and fractured the right leg above the ankle. She used crutches for walking after this. One month before entry she fractured the right humerus, and was confined to bed.

On physical examination the patient was a frail, short, little girl with rachitic deformities of the legs (see Fig. 6A). The right arm was in a Velpeau bandage. Ophthalmoscopic examination revealed no definite abnormalities. Dentition was retarded. The tonsils were large. The blood pressure was 100 mm. Hg systolic and 55 mm. diastolic. There were no manifestations to suggest the approach of puberty. The examination otherwise was of little interest. She measured 44½ inches in height and weighed 47.5 pounds.

Laboratory studies: In 100 cc. of serum there were 9.0 mg. of calcium (normal *circa* 10 mg.), 1.9 mg. of inorganic phosphorus (normal *circa* 4 mg.), 22.6 Bodansky units of phosphatase (normal *circa* 7 units for growing child), 6.5 gm. of protein, and 21 mg. of nonprotein nitrogen. The serum chloride concentration was 121 m.eq. per liter (normal *circa* 100 m.eq.), and the CO_2 combining power 19 m.eq. per liter (normal *circa* 26 m.eq.). Urine analysis: albumin +; sugar 0; sediment: 25 white blood corpuscles per high powered field. Urine culture: no growth. Phenolsulphonphthalein excretion after intravenous administration of 6.0 mg. of dye: 20 per cent in 15 minutes, 10 per cent more in 30 minutes, 15 per cent more in 1 hour. Red blood cell count 4,980,000; white blood cell count 9,000; oxygen capacity 18.7 vol. per cent; cell volume 43.5 per cent; blood smear not remarkable. Of the blood chemistry findings the following should be emphasized: the very low inorganic phosphorus level, the essentially normal serum calcium level, the very high phosphatase level, and the marked hyperchloremia with the decreased CO_2 content of the serum.

Roentgenographic studies showed extreme generalized decalcification, epiphyseal changes characteristic of rickets, evidence of old fractures, absent lamina dura about the teeth, and dense calcium deposits in the pyramids of both kidneys (see Figs. 1, 2, 3, 4).

Differential Diagnosis

In the diagnosis of the bone condition in our case and in that of Butler *et al.* (2) there were three possibilities which we considered: hyperparathyroidism with nephrocalcinosis, renal rickets combined with renal dwarfism, or some hitherto unrecognized condition.

Although the decalcification, the nephrocalcinosis, the hypophosphatemia, and the increased amount of phosphatase in the blood suggested hyperparathyroidism, that diagnosis seemed unlikely be-

cause of the normal serum calcium value. Only if there is a low serum protein level, or phosphate retention due to secondary renal insufficiency, may hyperparathyroidism be associated with a normal serum calcium value. Furthermore, as suggested elsewhere (3) and as confirmed by some unpublished data from this clinic, uncomplicated hyperparathyroidism in children does not cause epiphyseal changes. If the hyperparathyroidism is complicated by that form of renal insufficiency which one finds with renal rickets (*v. infra*), then epi-

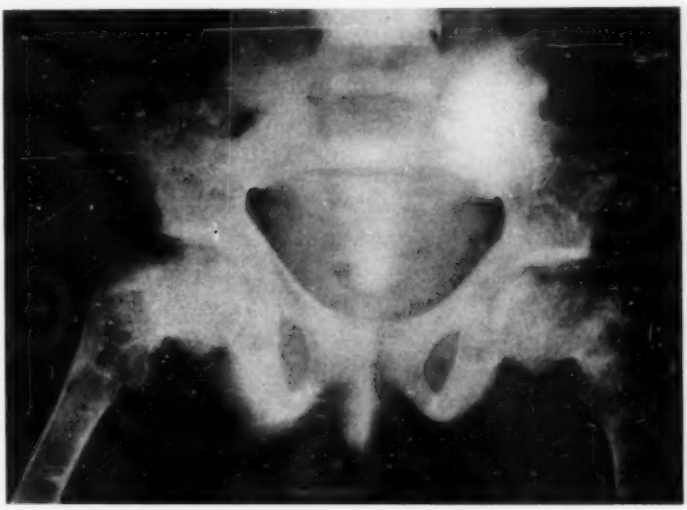


FIG. 1. ROENTGENOGRAM OF PELVIS ON JUNE 18, 1937, BEFORE TREATMENT
Note extreme decalcification

physeal changes suggestive of rickets may be present in the roentgenograms (3).

Renal rickets was likewise discarded as a diagnosis. It results from a different type of renal insufficiency than was present in this patient. There one finds phosphate retention with a tendency to secondary hypocalcemia, nitrogen retention, and a marked reduction in phenolsulphonphthalein excretion. Hyperchloremia is not a characteristic feature. This patient, on the contrary, had a diminished concentration of serum inorganic phosphorus, a normal concentration

of nonprotein nitrogen, a not very abnormal dye excretion as compared with other changes, a markedly increased concentration of serum chlorides, and a definite acidosis. As will be discussed below,

FIG. 2. ROENTGENOGRAM OF FEMURS ON FEB. 15, 1937, BEFORE TREATMENT

Note extreme decalcification of cortex of bone, wide epiphyseal lines, especially well demonstrated at upper end of left femur (*A*), and old fracture line at lower end of right femur (see arrows).

there was also fixation of the specific gravity and of the pH of the urine. Although glomerular function was definitely impaired (*v. infra*), it seemed that altered tubular function was the chief factor in the disturbed homeostasis.

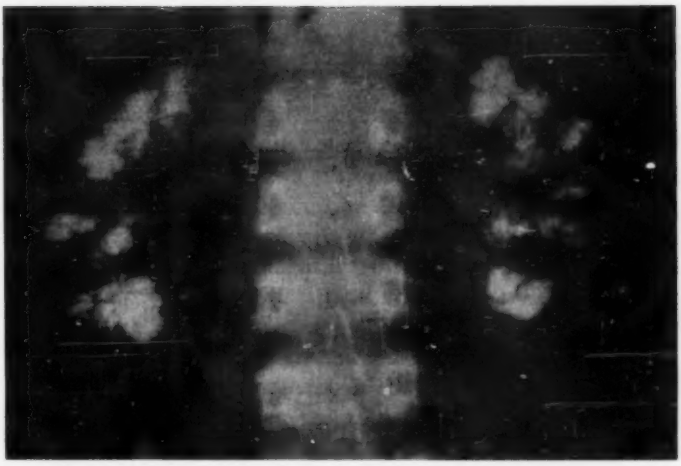


FIG. 3. ROENTGENOGRAM OF KIDNEYS SHOWING DENSE CALCIUM DEPOSITS IN PYRAMIDS OF KIDNEYS

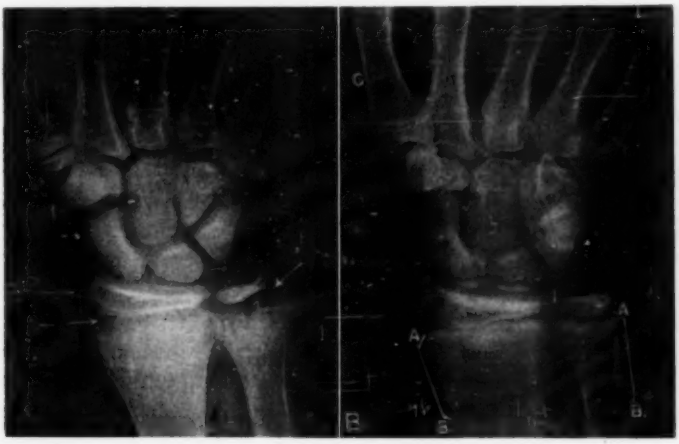


FIG. 4A. ROENTGENOGRAM OF WRIST TAKEN ON FEB. 15, 1937, BEFORE TREATMENT

Note rachitic changes in epiphyseal lines of radius and ulna (see arrows).

FIG. 4B. ROENTGENOGRAM OF WRIST TAKEN ON DEC. 31, 1937, AFTER TREATMENT

Note complete disappearance of rachitic changes in epiphyseal lines. Note dense regular trabeculae in zone A-B of normal bone which has been laid down since onset of therapy and compare with similar zones in Fig. 5. Note that growth line marked "C" has widened its distance from the epiphyseal line considerably.

The third possibility was some hitherto unrecognized syndrome. A syndrome secondary to the chloride acidosis seemed a possibility. Furthermore, the calcification of the kidney parenchyma might have been the cause of the chloride acidosis since it has been shown (2) that calcification of the tubules may be associated with a disorder which is characterized by a marked chloride retention. This has been verified in this clinic in patients with hyperparathyroidism associated with nephrocalcinosis (4). With an interpretation based on such a combination of considerations, the cause of the initial kidney lesion, of course, would still remain to be explained. One could hypothesize, perhaps, a healed pyelonephritis but this would be pure speculation. Regardless of how the tubules were damaged, the interpretation of the syndrome as due to a chloride acidosis resulting from the tubular changes seemed the most likely of the suggested possibilities. The chloride-acidosis-as-a-cause-of-the-rickets part of this thesis was susceptible to experimental proof; it offered clear-cut therapeutic indications.

Before the experimental data are discussed a few words should be mentioned concerning another possibility which is a modification of the above one and which cannot be completely dismissed. The chloride acidosis may have been due to some kidney lesion other than the calcification and may have caused both the rickets and the kidney calcification. Such an interpretation still leaves no explanation of the cause of the initial kidney lesion. The abnormality is probably not congenital for the patient was considered normal for her first 4 years. Furthermore, such an hypothesis takes no cognizance of the fact that there is considerable evidence that calcification in the kidney pyramids in instances where it is known that the calcification came first (e.g., hyperparathyroidism), is associated with chloride acidosis (*v. supra*).

PROGRAM OF METABOLIC STUDY

It was decided to do complete balance studies of calcium and phosphorus, and of the other electrolytes while the patient was on the following regimes:

A. Low calcium, neutral-ash diet with 5 grams of added NaCl daily. The purpose in this experiment was to obtain calcium and

phosphorus data which could be compared with those of other patients since this regime has been used extensively in this clinic with patients suffering from various conditions.

B. Moderately high calcium and phosphorus intake with 5 grams of added NaCl daily. The purpose here was to obtain control data for comparison with those from later regimes.

C. Same as B without the added NaCl. It was hoped that a restricted chloride intake would decrease the chloride acidosis and favor calcium deposition.

D. Same as C plus large amounts of vitamin D. This was to determine whether large amounts of vitamin D would have a favorable influence on the calcium balance.

E. A high calcium and phosphorus intake plus large amounts of vitamin D. This was a control for regime F.

F. Same as E plus a sodium citrate, citric acid mixture, which has been found effective by Hamilton and Dewar (5), and Shohl (6) in healing experimental rickets in rats, and by Shohl and Butler (7) in healing infantile rickets. The citric acid radicle not only acts as any other acid in the gastro-intestinal tract to lower the pH and favor calcium absorption, but by virtue of its being citrate, has a special action in dissolving calcium salts and thus promoting their absorption. In the body the citrate is mostly converted into CO_2 and H_2O, the sodium being left behind to act as base. The net result of such therapy, therefore, is to produce intestinal acidosis and blood alkalosis. The increased alkalinity favors calcium retention. This property seemed particularly desirable in this case since the approach which was being followed was based on the thesis that the rickets was due to the acidosis.

Collection of Samples and Methods of Analysis: The patient received a diet of similar composition on each day of a metabolic period. Except where a metabolic period followed directly on a previous one, the same diet was administered to the patient for at least four days before collecting the excreta. A duplicate diet for each period was analyzed directly for the separate constituents. The urine was collected in 24 hour amounts and preserved with toluol. The concentration of ammonia was determined within 24 hours; the other constituents at a later date. The stools were collected in 5-day periods, dried over a steam bath, and ground to a fine powder. Aliquots were taken for analysis.

The following methods of analysis were employed; sodium, Butler and Tuthill

(8); total base, Keys (9); potassium (10); calcium, Fiske and Logan (11); chlorides, Keys (12); CO_2 and oxygen, Van Slyke manometric method; protein, micro-Kjeldahl; phosphorus, Fiske and Subbarow (13); ammonia and urea, Van Slyke and Cullen (14); urine chlorides, Harvey (15); urine calcium, Fiske and Logan (16).

RESULTS OF METABOLIC STUDY

The metabolic data for calcium and phosphorus are shown in table I. During periods I, II and III (regime A) while the patient was on a low calcium, neutral-ash diet, the urinary calcium excretion (*circa* 0.16 gm. per day) was definitely elevated and there was a greater negative calcium balance than is normal for adults. The average daily urinary calcium excretion in normal individuals on the same regime is approximately 0.04 gm. and it is even less in growing children. These findings were consistent with the acidosis theory since the urinary calcium excretion is raised in acidosis.

During periods IV and V (regime B) the calcium intake was raised by giving 5 grams of calcium gluconate daily by mouth. Most of this calcium was excreted in the feces and the calcium balance still remained negative although considerably improved. It is of interest that this increased ingestion of calcium had no effect on the fecal phosphorus excretion.

During periods VI and VII (regime C) the 5 grams of added NaCl daily were withheld. The urinary calcium excretion fell from 0.18 gm. per day in period V to 0.14 gm. in period VI and to 0.11 gm. in period VII. The urinary phosphorus excretion, also, was lower in period VII than in any previous period. These findings suggested that the low chloride intake was associated with a decrease in acidosis and initiated a chain of favorable readjustments. One wonders whether positive calcium balances would have been obtained had this regime been continued for one more 5-day period.

In table II an opportunity is afforded to see in just what manner the low salt diet affected the acid-base metabolism. At the end of the high chloride regimes (A and B) the serum chloride level was 117 m.eq. per liter; after 10 days on the low NaCl regime (C) the chloride level was 112 m.eq. The corresponding values for bicarbonate were 13.8 m.eq. and 15.8 m.eq. respectively. Thus the chlorides fell 5 m.eq. and the CO_2 rose about 2 m.eq. per liter. Since the con-

TABLE I

TABLE I

Calcium and Phosphorus Metabolic Data

REGIME	PERIOD	DATE	CALCIUM, GRAMS AVERAGE PER DAY				PHOSPHORUS, GRAMS AVERAGE PER DAY				SERUM		
			Ur.	Fec.	Int.	Bal.	Ur.	Fec.	Int.	Bal.	Ca	P	Phosphatase
		1937									*mg.*	*mg.*	*Bodansky U.*
A. Low Ca, neutral-ash diet with 5 grams of added NaCl daily	I	Feb. 19–21	.15	.14	.10	−.19	.32	.17	.51	+.02	9.0	1.9	22
	II	24–28	.17	.13	.07	−.23	.36	.15	.53	+.02			
	III	March 1–5	.17	.14	.07	−.24	.38	.16	.50	−.04			
B. Moderately high Ca and P intake with 5 grams of added NaCl daily	IV	6–10	.18	.43	.53	−.08	.34	.15	.49	.00			
	V	11–15	.18	.45	.52	−.11	.35	.18	.51	−.02			
C. Same as B without the added NaCl	VI	16–20	.14	.44	.53	−.05	.38	.18	.56	.00			
	VII	21–25	.11	.48	.52	−.07	.33	.20	.53	.00			
D. Same as C plus 200,000 units of vit. D daily	VIII	26–30	.13	.37	.53	+.03	.30	.16	.54	+.08	9.7 I* 9.7 IV	2.5 2.9	27
	IX	31–Apr. 4	.14	.28	.53	+.11	.28	.13	.49	+.08	9.5 IV 10.9 V	2.8 2.8	
E. High Ca and P intake, low NaCl, 40,000 units of vit. D daily	X	April 17–21	.09	.35	1.07	+.63	.51	.25	1.23	+.47			
	XI	22–26	.10	.28	1.16	+.78	.64	.20	1.23	+.39			
F. Same as E plus Na "citrate mixture."	XII	May 10–14	.06	.30	1.13	+.77	.60	.20	1.32	+.52			
	XIII	15–19	.04	.15	1.04	+.85	.52	.13	1.18	+.53			

* Roman numerals indicate to which day of period blood data pertain.

TABLE II

Acid-Base Metabolic Data

REGIME	PERIOD	DATE	WEIGHT OF PATIENT	WHOLE BLOOD		SERUM—PER LITER								NH₃ IN URINE PER DAY	TOTAL FIXED BASE IN URINE PER DAY	SODIUM—M.EQ.; AV. PER DAY				POTASSIUM—M.EQ.; AV. PER DAY				CHLORIDE—M.EQ.; AV. PER DAY				NITROGEN—GRAMS			
				Ox. Cap.	Cell Vol.	Total Fixed Base	Na	K	Ca	Cl	Bicarbonate	Proteinate	"U.A."			Ur.	Fec.	Int.	Bal.	Ur.	Fec.	Int.	Bal.	Ur.	Fec.	Int.	Bal.	Ur.	Fec.	Int.	Bal.
		1937	*kg.*	*vol. per cent*	*per cent*	*m.eq.*	*m.eq.*	*m. eq.*	*m. eq.*	*m.eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m.eq.*	*m.eq.*															
		Feb. 9		18.7	42.6	147.8	138.7			114.1	12.8	15.8	5.1																
A. Low Ca, neutral-ash diet with 5 grams of added NaCl daily	I	19-21																											
	II	24-28	22.02											13.2	171	111	2	113	0	38.8	9.1	42.2	−5.7	109	3	123	11	6.3	.9	7.8	.6
	III	March 1-5	21.96											14.1	167	111	3	112	−2	38.4	9.0	38.6	−8.8	108	2	121	11	6.5	.8	8.0	.7
B. High Ca, and P intake with 5 grams added NaCl daily	IV	6-10	22.05		45.6	155.4 I*	144.1			122.0	12.9	16.8	3.7	14.2	167	109	1	112	2	38.5	5.1	41.4	−2.2	105	2	124	17	6.4	.6	7.0	0
		11	*22.21*											*13.2*	*188*	*118*				*40.3*				*111*				*6.2*			
		12	*22.12*											*13.0*	*174*	*111*				*39.8*				*104*				*6.7*			
		13	*22.12*											*13.4*	*181*	*116*				*38.2*				*113*				*6.5*			
		14	*22.22*											*11.2*	*176*	*111*				*38.6*				*106*				*6.5*			
		15	*22.25*											*22.4*	*169*	*112*				*40.6*				*109*				*6.2*			
	V	11-15	22.18											14.6	178	114	1	113	−2	39.3	6.0	40.0	−5.3	109	2	125	14	6.4	.8	7.5	.3
C. Same as B without the added NaCl		*16*	*22.21*			*150.7 I**	*142.5*			*117.0*	*13.8*	*16.8*	*3.1*	*15.4*	*138*	*75*				*44.0*				*73*				*6.8*			
		17	*21.91*											*14.1*	*111*	*44*				*47.0*				*48*				*6.9*			
		18	*21.73*											*13.4*	*98*	*31*				*45.4*				*38*				*6.9*			
		19	*21.82*											*17.0*	*93*	*30*				*46.1*				*32*				*7.4*			
	VI	20	*21.71*											*18.4*	*97*	*30*				*46.6*				*34*				*8.0*			

	VI	16 20	21.88		45.0										15.7	108	42	1	29	−14	45.8	6.0	38.8	−12.9	45	2	39	−8	7.1	.7	7.8	0
		21	*21.80*												*14.4*	*78*	*23*				*35.4*				*20*				*6.3*			
		22	*21.65*												*15.0*	*90*	*26*				*40.0*				*23*				*7.2*			
		23	*22.75*												*15.8*	*85*	*25*				*39.8*				*23*				*7.1*			
		24	*21.83*												*15.4*	*87*	*28*				*38.2*				*22*				*6.6*			
		25	*21.78*												*14.5*	*90*	*30*				*39.0*				*23*				*7.1*			
	VII	21-25	21.76												15.0	86	26	1	29	2	38.5	6.2	43.2	−1.5	22	2	36	12	6.9	.8	8.5	.8
D. Same as C plus 200,000 U. vitamin D daily	VIII	26-30	21.84		47.3	150.6 I*	140.6			111.6	15.8	16.8	6.4	15.8	93	24	1	26	1	35.2	6.7	46.3	4.4	21	2	36	13	6.5	.8	8.7	1.4
	IX	31-Apr. 4	22.08		45.6	150.7 V*	141.9		5.5	111.6	17.1	15.8	6.2	12.5	79	25	1	27	1	35.5	6.8	43.6	1.3	22	2	36	12	6.6	.9	7.6	.1
E. Very high Ca and P intake, low NaCl, 40,000 U. vitamin D daily	X	17-21	23.22		42.8	153.4 I*	141.4	4.2	4.2	115.5	14.4	15.3	8.2	15.4	134	50	5	52	−3	65.8	14.8	81.6	1.0	50	5	66	11	7.9	1.0	9.1	.2
	XI	22-26	23.36		42.9	153.2 V*	142.2	3.8	4.4	116.0	14.2	15.3	7.7	15.6	131	51	2	52	−1	59.1	9.5	77.4	8.8	47	3	64	14	7.4	.7	9.3	1.2
F. Same as E plus "citrate mixture"	XII	May 10-14	24.18		43.2	155.1 I*	144.1	3.2	5.1		20.1	15.3					4	59			7.8	71.4		55	3	64	6	7.3	.7	9.1	1.1
	XIII	15-19	24.48		43.3	152.8 V*	142.2	3.6	4.5	112.3	19.0	15.6	5.9				3	58			8.1	71.4		52	3	65	10	7.1	.6	9.4	1.7

* Roman numerals by serum total fixed base values indicate on which day of period blood was drawn.
** "U.A." = undetermined acid = total fixed base—(chloride bicarbonate proteinate).

centration of serum total base remained constant, there was a rise in the undetermined acid (U.A.). U.A. was taken as the difference between the total base and the sum of the determined acid radicles (chloride, total CO_2, and proteinate).

Examination of the balance observations shows that the patient was approximately in sodium equilibrium. Furthermore when added salt was withheld the balance for sodium reached equilibrium in about 4 days. In all periods except VI, the first one on a low salt diet, there was a small positive chloride balance. The chloride balance like that of the sodium rapidly reached its previous equilibrium point when the added salt was withheld. The positive balance was not lower on the low salt diet, once equilibrium had been reached, than on the high salt diet. It would appear, therefore, that a change from a high to a low salt intake produced a diminution of both serum chloride and acidosis (as shown by serum bicarbonate level). No explanation suggests itself for the positive chloride balances. No correction was made for chloride lost in the perspiration.

During periods VIII and IX (regime D) 200,000 U.S.P. units of vitamin D daily were added to the intake. This caused a marked reduction of the fecal calcium excretion without an appreciable change in the urinary excretion and the calcium balance became positive. The fecal phosphorus excretion was decreased to a lesser degree while the urinary phosphorus excretion was slightly lowered. These findings are in agreement with hypotheses as to the action of vitamin D discussed in a previous paper (17). Vitamin D had no significant effect on the metabolism of constituents other than calcium and phosphorus.

During periods X and XI (regime E) which were run after a lapse of 13 days the patient received a quite high calcium, high phosphorus, low NaCl diet plus 40,000 U.S.P. units of vitamin D daily. On this regime the fecal calcium and phosphorus excretions were surprisingly low which indicated excellent absorption and the urinary calcium excretions were the lowest yet obtained.

Regime F (periods XII and XIII) was the same as E except that the patient received 60 cc. of the *citrate mixture* daily by mouth. The mixture consisted of 140 gm. citric acid and 98 gm. sodium citrate in 1000 cc. of water. On this regime the absorption of calcium and phosphorus was apparently even further increased, and the urinary

excretion of calcium was definitely further decreased. In period XIII the urinary calcium excretion reached the lowest level observed, .04 gm. per day. The effect of the *citrate mixture* would probably have been more convincing if the balances had not already been so favorably influenced by the large doses of vitamin D and the after-effect of the even larger doses during periods VIII and IX. In table II it may be seen that this regime did alleviate the acidosis, the bicarbonate of the serum rising from 14.2 m.eq. to 20.1 m.eq. per liter.

In order further to test the patient's ability to excrete chloride, she was given on April 5, 1937, seven grams (130.7 m.eq.) of NH_4Cl. On the following morning her serum chloride level had risen from 111.6 to 123.5 m.eq. per liter, and her serum bicarbonate had fallen from 18.4 to 8.5 m.eq. per liter. The other serum electrolytes showed only minor changes. With this acidosis the urine pH fell only to 6.0.

In February 1938, at a time when the bone condition had been entirely cured, as will be discussed below, these tests were carried further. The patient received a diet of the same low salt composition each day. After six days on this diet the serum electrolyte concentrations were normal (see table III) except for a high serum chloride value (110.9 m.eq. per liter) and a low serum bicarbonate value (19.8 m.eq. per liter). Eight grams of NaCl daily were then added to the diet. After 3 days on this regime there was a rise (6.7 m.eq.) in the chloride concentration which was offset by a 2.1 m.eq. fall in the bicarbonate, a 2.3 m.eq. rise in the total base, a 0.7 m.eq. fall in proteinate, and a 1.6 m.eq. fall in "undetermined acid." This seemed to indicate that the ingestion of NaCl increased the chloride acidosis. The added NaCl was then omitted and 90 cc. daily of the *citrate mixture* was administered. After 4 days on this regime the chloride fell 12.7 m.eq. to a value 6.0 m.eq. lower than had been obtained by the low salt diet alone (*v. supra*). The bicarbonate rose 8.5 m.eq. to 26.2 m.eq. per liter.

If these observations are taken in conjunction with the previous ones it is quite clear that the administration of chloride without fixed base (*cf.* NH_4Cl experiment) caused a marked chloride acidosis, that the administration of chloride with an equivalent amount of sodium (NaCl) caused a moderate chloride acidosis, and finally that the

administration of sodium without chloride (*citrate mixture*) alleviated the chloride acidosis.

The metabolic data seemed to indicate, therefore, that a low chloride, high calcium, high phosphorus, high vitamin D regime plus the *citrate mixture* was indicated as far as the skeletal part of the syndrome was concerned. It was hoped that the drawfism might be alleviated as the acidosis decreased. It was recognized that any increase in alkalosis might favor calcium deposition in the kidneys, but it was felt

TABLE III

Serum electrolyte studies showing the effect on the chloride acidosis of NaCl in the diet and of the "citric acid mixture"

TREATMENT OF PATIENT PRIOR TO OBTAINING OF BLOOD SAMPLE	DATE	SERUM—M.EQ. PER 1000 CC.								SERUM PHOSPHORUS
		Total Fixed Base	Na	Ca	K	Cl	Bicarbonate	Proteinate	U.A.	
	1938									*mg. per 100 cc.*
After 6 days on measured low NaCl diet	3/4	154.0	142.3	4.7	3.2	110.9	19.8	15.6	7.7	4.1
After 3 days without further change except for addition of 8 gm. of NaCl daily	3/7	156.3	143.3	4.6	5.0	117.6	17.7	14.9	6.1	4.0
After 4 days of same regime without added NaCl and with "citric acid mixture"	3/11	154.5	143.6	4.4	3.3	104.9	26.2	14.6	7.5	4.1

* "U.A." = total base—(Cl plus bicarbonate plus proteinate). Phosphorus, though determined, was disregarded.

that this chance had to be taken. Furthermore, under such a regime the calcium excretion in the urine was markedly diminished which would be a factor against the possibility of further kidney destruction.

RESULTS OF TREATMENT

The patient was discharged to the Bradley Home in May 1937, where the therapeutic measures were started. She received, three times daily, two glasses of milk, five drops of Squibb's *Navitol*[1] and

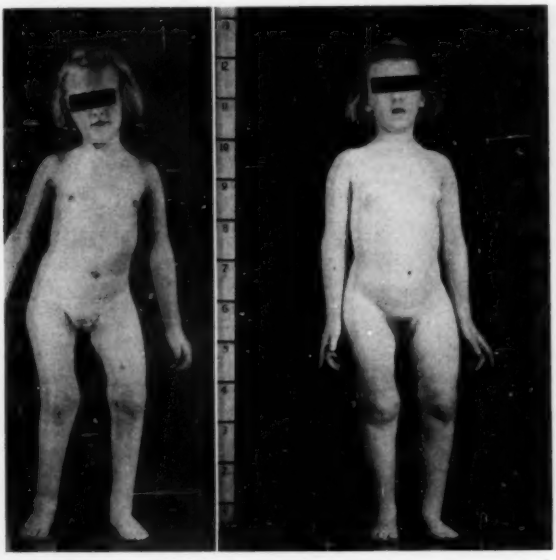
[1] 1 gram of *Navitol* contains 10,000 vitamin D units.

FIG. 5. ROENTGENOGRAM OF PELVIS AND FEMURS ON DEC. 31, 1937, AFTER SIX MONTHS OF TREATMENT

Contrast with Figs. 1 and 2. Note increased density of bone and increased thickness of cortex. Note, at upper end of femur, zone (*A*) of thick regular trabeculae which represents bone formed by epiphyseal cartilage since beginning of treatment and which demonstrates not only complete alleviation of rickets but rapidity of growth. Note rim of new periosteal bone around pelvis, seen especially well at *B*.

20 cc. of the sodium citrate, citric acid mixture (*v. supra*). No added NaCl was allowed. It should be emphasized that the dose of vitamin D was very moderate compared to that used in the metabolic experiments and that it is unlikely that the extraordinary therapeutic results were attributable to it.

The results were almost miraculous. When she was seen in December 29, 1937, seven months later, she had completely recalcified her



A B

FIG. 6A. PHOTOGRAPH OF PATIENT ON DEC. 3, 1936, BEFORE TREATMENT
FIG. 6B. PHOTOGRAPH OF PATIENT ON FEB. 29, 1938, AFTER TREATMENT

skeleton (Fig. 5). All roentgenographic evidence of active rickets had disappeared (Fig. 4B). The serum phosphorus level had risen to 5.3 mg. per cent; the serum phosphatase level had fallen to 14.4 Bodansky units. From a frail, bed-ridden child she had developed into a sturdy, still under-sized, young woman (Fig. 6B). Her weight had increased from 25.5 kg. to 31.0 kg. Her breasts had developed and she had had her first menstrual period. She had grown 3 inches

during the previous five months, whereas during the preceding eight months she had grown less than one inch. Roentgenograms of her skeleton showed the rapidity of her growth very well, owing to the fact that the normal bone laid down in juxtaposition to the epiphyseal lines since the treatment had started, contrasted very strikingly with the bone laid down before treatment (Figs. 4B and 5). Her serum chlorides were only slightly elevated, 107 m.eq. per liter. Though higher than before treatment (13.5 m.eq.), her total CO_2 was still low, 19.6 m.eq. per liter. The oxygen capacity of her whole blood had risen from 18.7 vol. per cent to 20.3 vol. per cent. Furthermore, it appeared that her kidney lesion had improved. Roentgenograms of the kidneys showed some decrease in the calcium deposits. The patient had passed a few calculi from time to time. Her urine now contained no albumin and fewer white cells. The specific gravity of the urine continued to be fixed at about 1.010, however. The pH of her urine, likewise, varied only between 6.5 and 7.0.

PATHOLOGICAL PHYSIOLOGY OF KIDNEY

The first question was whether glomerular function was comparatively little impaired as suggested by the normal non-protein nitrogen level and the low blood phosphate level. In table IV the results of simultaneous inulin, creatinine, and urea clearances are given (18). The average inulin clearance was 58 cc. per min. corrected to 1.73 sq.M. body surface (lower limit of normal *circa* 90 cc.). This indicated a significant but not extreme diminution of glomerular filtration. The creatinine and urea clearances were likewise impaired. One may conclude, therefore, that the kidney disease was more widely spread than it had appeared to be at first.[2] The more marked functional impairment, none the less, was in the tubules. The inability to acidify and concentrate the urine pointed to an abnormality in the distal tubule, if one is to accept for the mammalian kidney the work of Montgomery and Pierce (19), Walker, Hudson, Findley, and

[2] This statement is made with some reservation. When the renal tubules are not functioning normally there is no assurance that inulin clearance accurately measures glomerular filtration. Part of the inulin which has passed through the glomerular membrane may return to the blood stream through the walls of the damaged tubules.

Richards (20), and Walker and Hudson (21, 22) on the kidney of amphibians. A second urine concentration test done in 1938 by the method of Lashmet and Newburgh (23) showed the specific gravity fixed between 1.009 and 1.010 (see table V). The ammonia excretion (see table II), also a tubular function, was likewise low. Furthermore, even on the administration of the seven grams of ammonium chloride

TABLE IV

*Simultaneous Renal Clearances**

DATE	PERIOD	URINE FLOW	INULIN†		CREATININE		UREA		SODIUM		CHLORIDE		INULIN URINE TO PLASMA RATIO	REMARKS
			Plasma	Cc. plasma cleared per min.	Plasma	Cc. plasma cleared per min.	Plasma	Cc. plasma cleared per min.	Plasma	Cc. plasma cleared per min.	Plasma	Cc. plasma cleared per min.		
1938		*cc./min.*	*mg. per cent*		*mg. per cent*		*mg. per cent*		*mg. per cent*		*mg. per cent*			
11/26	I	7.8	250	54	19.8	99	76.6	41	316	1.08	399	2.32	4.4	Low salt diet—1.5 gm. per day during previous three days
11/30	I	8.4	243	50	21.4	93			318	5.43	421	6.15	3.8	High salt diet—19.5 gm. per day during previous three days
	II	9.2	190	65	19.2	93			318	5.82	416	7.40	4.6	
Average				58		93				5.60		6.78		
12/16	I	11.2	164	57	14.2	90			331	2.14	415	2.64	3.3	"Citrate mixture" during previous three days
	II	5.3	146	48	13.2	78			327	1.58	413	1.94	5.9	
	III	4.1	140	57	11.2	95			328	1.83	411	2.25	9.0	
	IV	2.7	115	55	10.6	85			327	1.49	411	1.83	13.1	
Average				54		87				1.76		2.16		

* All clearances corrected to surface area of 1.73 sq.M. Surface area of M. J. = 1.118 sq.M.

† Approximately 90 minutes before collection of urine and blood, 800 cc. of fluid were given by mouth. 15 minutes before collection 20 gm. of inulin were given rapidly by intravenous infusion. An additional 10 gm. were given by slow infusion throughout the collection period.

on April 5, 1937, when the serum bicarbonate went from 18.4 to 8.5 m.eq. per liter, there was only a slight rise in the ammonia excretion (from 11.2 m.eq. on April 4 to 15.5 m.eq. on April 5). Finally, the calcification in the pyramids, shown in the roentgenogram, suggested tubular rather than glomerular disease although it is recognized that the pyramids are composed mostly of the relatively non-functioning collecting tubules.

The second question was whether the chloride retention represented a specific inability of the kidney to excrete chloride radicles or whether the same would be true of all mineral acids. Accordingly some experiments were performed in which the effect on the serum chloride and bicarbonate levels of ammonium chloride ingestion was compared with the effect of ingestion of an equivalent amount of ammonium nitrate. These experiments showed that the ammonium nitrate likewise caused a marked lowering of the CO_2. This would seem to indicate that the chief functional incapacity of this patient's kidneys was that of excreting acid, not just chloride radicles.

Our final interpretation of the pathological physiology of the kidney, therefore, is somewhat as follows. The patient's kidneys, because of predominantly tubular disease, have a decreased ability to make ammonia or to excrete an acid urine and hence are at a disadvantage

TABLE V

Urine Concentration Tests

TIME	URINE VOL.	ALBUMIN GM. PER LITER	SPECIFIC GRAVITY*
	cc.		
11/17/38 7 a.m.–11/18/38 7 a.m.	980	0.29	1.010
11/18/38 7 a.m.–11/18/38 9 a.m.	108	0.29	1.010
11/18/38 9 a.m.–11/18/38 11 a.m.	103	0.43	1.009

* Specific gravities corrected for temperature and albumin.

in warding off an acidosis. Mineral acids to be excreted require an equivalent amount of base. With an excess of chloride or nitrate radicles to be excreted, there are only two possibilities of adjustment. Either fixed base can be withdrawn from body fluids or the chloride or nitrate radicles can be withheld. The latter adjustment apparently predominates in this case. The *citrate mixture* works by furnishing an excess of fixed base for excretion purposes.

The question has been raised as to why the ingestion of the neutral substance, NaCl, causes an acidosis. Dr. John E. Howard made an interesting suggestion. He pointed out that the ratio of Na to Cl in NaCl is 1:1; that the ratio in normal glomerular filtrate is presumably the same as that in the blood, namely 1.4:1.0; that, therefore, if the function of the tubules was markedly deficient, there would be a tendency to lose from the body more Na than Cl.

CAUSE OF RICKETS

The next question is why the chloride acidosis in this instance caused rickets whereas a chloride acidosis due to the administration of ammonium chloride to a normal individual leads to osteitis fibrosa (24). It might be well to say a word about the difference between the two conditions. Bone formation and bone resorption proceed continuously side by side. Osteitis fibrosa is due to a primary increase in bone resorption and is accompanied by a secondary increase in bone formation. Rickets, on the other hand, is associated with a failure of calcium deposition where bone is being formed. Any acidosis increases bone resorption (e.g., acidosis due to ammonium chloride ingestion). For lack of calcium deposition it is usually necessary that the calcium ions or phosphate ions or both be depressed in the serum. In a recent paper from this clinic (17) vitamin D deficient rickets was ascribed to the following sequence of events: vitamin D deficiency → lack of calcium absorption from gastrointestinal tract → tendency to hypocalcemia → secondary hyperparathyroidism to meet this tendency → low serum phosphorus level → rickets due to low serum phosphorus with normal serum calcium. In the situation under discussion it is believed that the final steps are the same, but that, instead of there being a decreased calcium absorption into the blood, there is an increased loss of calcium from the blood into the urine because of the demand for calcium as a fixed base to help in the excretion of acid radicles. The chief beneficial effect of the *citrate mixture* in our patient, it is believed, was to furnish sodium for excretion in the urine, thus sparing calcium (cf. in table I calcium excretion in urine of 90 and 100 mg. per day in periods X and XI and of 60 and 40 mg. in periods XII and XIII). Vitamin D probably worked by increasing calcium absorption and thus increasing the base available for excretion; it is probably significant that vitamin D did not decrease the urinary calcium excretion (compare period VIII and IX with VI and VII, table I).

CAUSE OF DWARFISM

An interesting feature of this case is the restoration of growth. One is tempted to attribute this to the reduction of the acidosis. "Renal rickets" also has dwarfism as one of its features and also

has an accompanying acidosis. Marked restoration of growth with alkali therapy in a case of renal rickets has been reported by Graham and Oakley (25). In that condition, however, it is not always possible to overcome the acidosis without precipitating tetany because of the accompanying hypocalcemia resulting from the high serum phosphate level.

SIMILAR (?) CASES FROM LITERATURE

Although it is quite clear that the patient here described and the patient of Butler *et al.*, suffered from the same disease entity, characterized by rickets, dwarfism, chloride acidosis, hypophosphatemia, and calcification of the renal pyramids, it is not beyond question that this entity is fundamentally different from previously described conditions. That it is different from ordinary renal rickets is certain and has already been discussed. However, there have been at least two cases (25, 27) of renal disease associated with rickets in which low blood phosphorus levels have been present. Such, indeed, is the rule in that interesting condition, de Toni-Fanconi disease, a complex characterized by low phosphorus rickets, renal glycosuria, acidosis caused by an unidentified organic acid (not HCl) and dwarfism (see Eliot and Park (28) for discussion and literature). To be sure in none of these other cases was any mention made of hyperchloremia or of calcification of the kidney pyramids. However, as discussed above, the evidence is not yet completely clear that this calcification is the first step in the disease and hence an integral part; it is possible that it is a result of the disease and thus not a fundamental characteristic. The chloride acidosis, on the other hand, has been proved to be an integral part of the disease. Cases of rickets not due to vitamin D deficiency, and not renal rickets in the usual sense, have been reported in which the rickets was improved by alkalis (29, 30, 31). A chloride acidosis was not reported in these cases nor was evidence presented that the acidosis was on a renal basis. That the usual form of renal rickets is associated with an acidosis and is improved by alkali therapy (25) is hardly relevant to the present discussion.

Abstract of Similar Case Reported by Butler, Wilson and Farber (2)

The following quotation is taken from the paper by Butler *et al.*

M. L. was a ten-year-old boy admitted to the hospital because of weakness and stunted growth. He developed "rickets" at one and a half years of age. At four years he fell and broke his right femur and from that time failed to grow normally. Weakness, polydipsia, and polyuria became marked during the pre-

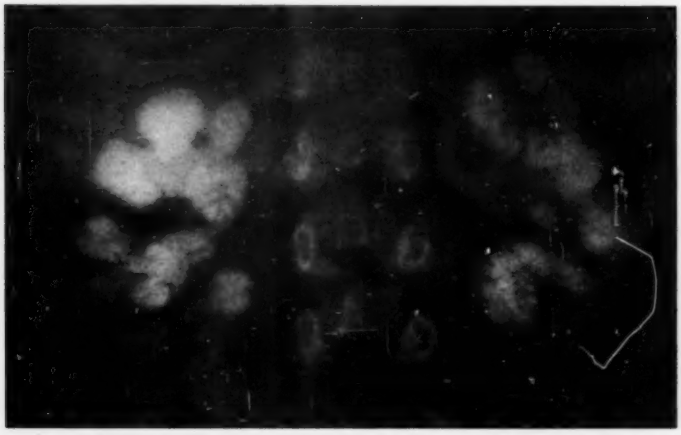


FIG. 7. ROENTGENOGRAM OF KIDNEYS OF CASE OF BUTLER ET AL. (2)
Compare with Fig. 3

FIG. 8. ROENTGENOGRAM OF WRIST OF CASE OF BUTLER ET AL. (2)
Note evidence of florid rickets. Compare with Fig. 4A.

ceding two years. Examination showed an underdeveloped and moderately emaciated boy who had difficulty in raising himself from the prone to standing position. The urine contained varying amounts of albumin and casts and numerous red and white blood cells. Its specific gravity was limited to 1.009. The

blood pressure was 110 over 75. Roentgenograms showed general osteoporosis and broad osteoid zones at the ends of the long bones, as seen in rickets tarda or renal rickets, and diffuse calcium deposits in both kidneys (Figs. 7, 8, 9). Phenolsulphonphthalein excretion was 70 per cent in two hours. Serum analyses showed normal serum calcium, low inorganic phosphorus, elevated chloride, a low bicarbonate, normal N.P.N. concentrations, and a phosphatase concentration of 1.0 (normal 0.3).[3] The low serum phosphorus level and the adequacy of the kidney function were not consistent with renal rickets. Roentgenograms showed no cystic areas in the bones. Moreover, inasmuch as the urinary excretions of

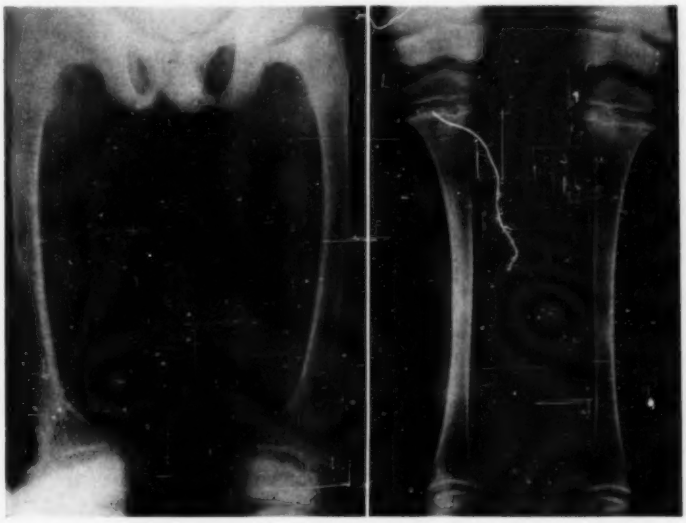


FIG. 9. ROENTGENOGRAMS OF LOWER EXTREMITIES OF CASE OF BUTLER ET AL. (2)
Note wide epiphyseal lines, bending deformities, and extreme decalcification.

calcium and phosphorus were but slightly above the normal levels, balance data did not support the diagnosis of hyperparathyroidism without a severer kidney insufficiency than seemed present in this case. Nephrolithotomy was attempted on one kidney, but the calcification was so diffuse and intimately associated with the renal parenchyma that its removal was impossible without excessive damage to the renal structure. The opaque material removed was shown on analysis to be a calcium phosphate carbonate compound of the composition of bone. During eight weeks the serum chloride varied between 124 and 114 m.eq. per liter and the

[3] Kay Units.

serum bicarbonate never exceeded 16 m.eq. per liter. The patient left the hospital against advice before a diagnosis was made.

SUMMARY AND CONCLUSIONS

The case history of a girl of thirteen with persistent rickets and dwarfism, is presented. The roentgenograms and the blood chemistry—normal serum calcium, low serum phosphorus, and high serum phosphatase—indicated that the condition was rickets and not osteitis fibrosa. The condition was accompanied by massive calcium deposits in the pyramids of the kidneys, hyperchloremia, and a low serum bicarbonate level.

An almost identical case history from the literature is reviewed. The condition is considered a disease entity.

Kidney function tests showed only a moderate decrease of inulin clearance, markedly decreased ability to excrete chlorides and to make ammonia, and a fixation of the specific gravity and pH of the urine.

The chloride acidosis was markedly increased by the ingestion of chloride without fixed base (NH_4Cl); was somewhat increased by the ingestion of chloride with fixed base (NaCl); and was decreased by the ingestion of fixed base without chloride (sodium citrate and citric acid mixture). Ammonium nitrate was equally efficacious, equivalent for equivalent, with ammonium chloride in depressing the serum bicarbonate level.

The following sequence of events is proposed as the cause of the rickets: (1) inability to make ammonia or to excrete an acid urine due to renal tubular disease; (2) shortage of base with which to excrete mineral acids, notably chloride radicles; (3) increased loss in the urine of calcium acting as a base; (4) tendency to a low serum calcium level; (5) secondary hyperparathyroidism to meet this tendency; (6) hypophosphatemia; (7) "low phosphorus" rickets.

Metabolic studies showed that the calcium and phosphorus balances were improved by a low NaCl diet, more so by vitamin D, and probably even more so by a mixture of sodium citrate and citric acid. The low NaCl diet and especially the *citrate mixture* produced an elevation of the serum CO_2 and a lowering of the serum chloride level.

Therapeutic measures, based on the results of the studies, were tried; they consisted of a low salt diet, only moderate amounts of vitamin D, and the ingestion of the *citrate mixture*. There resulted an extraordinary improvement of the bone disease. The therapy also lead to a reestablishment of growth. It is suggested that the failure to grow may have been due to the acidosis.

The condition is contrasted with "renal rickets" which is not rickets but osteitis fibrosa, and which is associated with a form of kidney disease exhibiting notably phosphate and nitrogen retention.

It is suggested that the condition here described, characterized by rickets with hypophosphatemia, dwarfism, calcification of the pyramids, and chloride acidosis, be designated "nephrocalcinosis with rickets and dwarfism."

The authors wish to take this opportunity to thank Dr. Charles Bradley for his cooperation in arranging for the studies and in seeing that the treatment was carried out, Dr. Alfred T. Shohl for suggestions in regard to the *citrate mixture*, Dr. Allan M. Butler for suggesting that the *citrate mixture* be used and for allowing us to publish the x-ray photographs of his patient, Dr. James Bordley III for suggestions as to the pathological physiology of the kidney, and Dr. Edwards A. Park for helpful criticisms.

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THE MEDICAL AND SURGICAL TREATMENT OF SEVERE BRONCHIAL ASTHMA[1]

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Patients with mild bronchial asthma usually are symptomatically relieved by single or repeated oral administrations of ephedrine sulphate. When ephedrine sulphate is ineffective, or when it cannot be administered for reasons peculiar to the patient, the inhalation of epinephrine hydrochloride (1:100) from a specially prepared atomizer is frequently effective.

Patients with severe bronchial asthma rarely receive relief from either of these therapeutic measures and it is this group of asthmatic patients that becomes a stumbling block to physicians who are not daily confronted with the problems of treating bronchial asthma. This situation has arisen not because the problem is particularly difficult but because the literature on the subject has been divergent.

A discussion of the medical and surgical treatment of patients with severe bronchial asthma may be simplified by dividing the patients into 3 groups, viz., (1) patients with severe acute bronchial asthma, (2) patients with intractable asthma ("status asthmaticus"), and (3) patients with severe chronic bronchial asthma.

TREATMENT OF PATIENTS WITH SEVERE ACUTE BRONCHIAL ASTHMA

Epinephrine hydrochloride (1:1000) administered subcutaneously in doses of 0.3 to 0.5 cc. is usually effective in relieving, at least temporarily, a severe attack of asthma. Occasionally, it is necessary to administer repeated injections at 15 minute intervals. In a small group of patients, asthma persists despite intensive epinephrine ther-

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apy and these patients are described as having intractable asthma. As a general rule, one may consider the asthma to be intractable if relief is not obtained from 4 injections of epinephrine hydrochloride given at 15 minute intervals. The treatment of intractable asthma will be considered later.

Although epinephrine hydrochloride is therapeutically beneficial in giving immediate relief from asthmatic symptoms, its duration of effectiveness is brief. This is so, because epinephrine hydrochloride is rapidly absorbed and destroyed after its administration.

It is the rule, rather than the exception, for severe asthma to recur. Such recurrence of symptoms exaggerates the initial insult to the respiratory tree and prolongs the period of illness. Therefore, any therapeutic measure which will prevent recurrence of asthma and allow the patient rest and relaxation is highly desirable. The use of epinephrine-in-oil, a slowly absorbed preparation of epinephrine, is such a therapeutic measure.

Since the isolation of epinephrine by Abel (1) many investigators have attempted, by modifying the original structure of the epinephrine molecule, to produce compounds with more prolonged activity. However, because the molecule is small and very labile, the problem has been difficult. Recently (2), it has been demonstrated that a suspension of powdered epinephrine in peanut oil, so prepared that 1.0 cc. of oil contains 2 mg. of epinephrine, is slowly absorbed and has a prolonged epinephrine effect.

The effectiveness of epinephrine-in-oil in the treatment of acute bronchial asthma, is well established (2). The dose is regulated according to the severity of the asthma and the patient's tolerance for epinephrine. As a general rule, 0.75 to 1.0 cc. is a sufficient and safe dose. In very severe asthma larger doses may be necessary.

Because epinephrine-in-oil is slowly absorbed, its effect is not manifest for 15 to 30 minutes after its injection. For this reason, it is advisable to administer beforehand an injection of 0.3 to 0.5 cc. of epinephrine hydrochloride (1:1000) to provide immediate relief of symptoms. Such a dose may be repeated every 15 minutes until relief is obvious or until 4 injections have been administered. When symptomatic improvement is apparent, an intramuscular injection of 0.75 to 1.0 cc. of epinephrine-in-oil should be given. If relief is

not obtained from a single or repeated injections of epinephrine hydrochloride it is useless to administer epinephrine-in-oil.

The final step in treating the patient is the administration of a sedative. Any one of the various barbituric acid derivatives may be employed. If asthmatic symptoms have persisted a day or so and rest must be guaranteed, 0.3 to 0.5 cc. of Schlesinger's solution[2] is permissible.

It is pertinent in a discussion of the medical treatment of asthma to comment on the cardiovascular response to epinephrine, and its use in asthmatic patients with hypertension. Clough (3) and Lyon (4) have demonstrated that the cardiovascular response to 1 mg. of epinephrine hydrochloride injected subcutaneously, varies in different individuals and even in the same individual at different times. In the majority of patients a maximum rise in systolic blood pressure of 30 mm. Hg is obtained in 5 to 20 minutes following the injection. There is usually a concomitant tachycardia, with a fall in diastolic pressure of 5 to 15 mm. Hg. The entire reaction is over in 45 to 90 minutes. Keeney and others (2) noted that the cardiovascular response to epinephrine-in-oil lasted for about 9 hours. Fortunately, larger doses than are used in the treatment of asthma, were necessary to produce a rise in systolic pressure of 20 to 30 mm. Hg.

There is evidence (5) that patients with hypertension are not abnormally sensitive to epinephrine. On the other hand, there is evidence that an increase in the pulmonary CO_2 tension (6), exercise (7), and various psychic disturbances (8) may produce a rise in the blood pressure of hypertensive individuals. The asthmatic state incorporates all three of these factors. Theoretically, therefore, there is adequate cause for an increase in the blood pressure of hypertensive asthmatics if the dyspnea is allowed to persist. With these facts in mind, it becomes obvious that hypertensive asthmatics must be relieved of their asthma and that the cautious use of epinephrine is not contraindicated. In my clinical experience there has been a fall of the blood pressure in such patients, concomitant with the onset of the relief from asthma, brought about by epinephrine.

[2] 0.5 cc. of Schlesinger's solution contains 0.000125 gm. scopolamine hydrochloride, 0.01 gm. morphine hydrochloride, 0.015 gm. dionin (ethylmorphine hydrochloride).

TREATMENT OF PATIENTS WITH INTRACTABLE ASTHMA

The patient with intractable asthma ("status asthmaticus") presents an acute medical emergency for which there is no entirely effective therapeutic measure. Many different schemes of treatment, wholly unrelated, have been suggested as being worthy of trial. Any form of therapy that will relieve the grave respiratory distress immediately, even though only occasionally or temporarily, may be life saving.

The bronchi and bronchioles, in bronchial asthma, are partially obstructed by exudate, by muscular contraction, and by swelling of the mucosa. This partial obstruction is more marked in intractable asthma. The difference in degree of obstruction is probably due to an increase in the exudate and in the swelling of the mucosa. Therapy should be directed toward the alleviation of the obstruction and the assurance of adequate pulmonary ventilation.

It is necessary for the patient to relieve himself of the exudate by coughing. Epinephrine, because of its constrictive effect on the blood vessels, and its dilating effect on the smooth musculature of the bronchial tree, affords relief in the ordinary attack of asthma. Epinephrine is inefficient in intractable asthma and the patient, therefore, is commonly considered to be "epinephrine-fast." However, it is more logical to assume that the bronchial and bronchiolar obstruction has developed to such a degree that epinephrine alone is insufficient.

The mucosal swelling should be lessened by increasing the osmotic pressure in the circulating blood with some hypertonic solution, thereby drawing the excess fluid from the bronchial mucous membranes into the blood stream. For this purpose a hypertonic solution of glucose, sodium chloride, or sucrose may be employed. The osmotic effect of such substances as glucose and sodium chloride, which pass through the capillary wall with ease, is curtailed by diffusion. Sucrose, because its molecules are larger and less diffusible, remains in the blood stream a longer time after injection. Sucrose, furthermore, is not broken down to any significant extent or utilized as is glucose; nor is it stored in the tissues as is sodium chloride (9).

Masserman (10) studied the effects of the intravenous administration of 50 per cent sucrose in 35 normal patients and, according to

his results, its injection, at the rate of 5 to 10 cc. a minute in amounts no greater than 500 cc., is a safe procedure.

Keeney (11) administered intravenously 100 cc. of 50 per cent sucrose, to which was added 0.5 cc. of 1:1000 epinephrine hydrochloride, to patients with intractable asthma and obtained relief of symptoms for 1 to 3 hours. Although asthma recurred, the symptoms were adequately controlled by injections of epinephrine hydrochloride. Prior to the administration of the sucrose and epinephrine this had not been possible.

A 20 cc. syringe, to which is attached a three-way stop-cock, is used to administer the sucrose and epinephrine. A 20-gauge needle is attached to the vertical arm of the stop-cock, and a short piece of rubber tubing, which has previously been treated with sodium bicarbonate and acetic acid, is attached to the horizontal arm of the stop-cock. It is through this tubing that the solution is withdrawn from its container into the syringe. Thus, during the course of the administration the syringe can be refilled without withdrawing the needle from the vein. Because 50 per cent sucrose is very viscous, it cannot be given by the gravity method.

Untoward reactions following the administration of sucrose and epinephrine, such as carpo-pedal spasm, pain behind the eyes, nausea, and nervousness, have occurred only when the mixture was administered too rapidly.

Van Leeuwen (12) in 1933, first suggested the use of aminophyllin (theophylline ethylene diamine) for patients with intractable asthma; and three years later, Efron (12) stopped an attack of asthma, which had been refractory to epinephrine, by administering aminophyllin intravenously. The first extensive clinical report on the effectiveness of intravenous aminophyllin in the treatment of intractable asthma was published by Hermann and Aynesworth in 1937 (13). They administered 0.48 gm. of aminophyllin diluted in 10 cc. of normal sodium chloride solution to 16 patients on 41 different occasions. Thirty-one of the 41 injections afforded prompt, complete, and persistent relief. The latter authors suggested that the injection be made slowly. Reactions such as a feeling of heat, a burning in the eyes, a sense of constriction in the chest, nausea and vomiting, and, at times, convulsions and coma were reported. Efron (12) stressed

the fact that the solution may sclerose veins or cause excruciating pain, should it get under the skin. It is important to emphasize that the mode of action of aminophyllin on the respiratory tree has not been scientifically established. Be this as it may, the results obtained by this method of treatment have been sufficiently encouraging to warrant its empirical usage.

Patients with intractable asthma, in whom the bronchial and bronchiolar obstruction is extensive, develop signs of carbon dioxide retention and anoxia (14). The degree of cyanosis and polycythemia roughly parallels the degree of oxygen unsaturation of the arterial blood. The cyanosis is often enhanced by a dilatation of the peripheral capillaries and venules. The slowing of the general circulation may, in turn, intensify the anoxia and carbon dioxide retention.

Anoxia causes the respiratory center to be more sensitive to changes in the hydrogen-ion concentration of the blood. Hyperpnea represents an attempt on the part of the body to rid itself of excess carbon dioxide. This is not immediately accomplished in patients with intractable asthma and as a result the respiratory distress becomes violent. If anoxia persists there is finally exhaustion of the respiratory center with concomitant development of rapid and shallow breathing. As shallow breathing is not only a result but also a cause of oxygen want a dangerous cycle is produced (14).

The inhalation of an atmosphere of 100 per cent oxygen is usually effective in combating anoxia. Barach (15) has demonstrated that a mixture of 80 per cent helium and 20 per cent oxygen is inspired and expired with greater ease than an atmosphere of 100 per cent oxygen. Therefore, it is conceivable that the state of oxygen want may be relieved more satisfactorily by employing helium and oxygen.

In bronchial asthma there is inspirational as well as expirational difficulty. Moore and Binger (16) and Kernan and Barach (17) have demonstrated that when dogs are made to inspire against resistance, there develops a progressive increase in the intrapleural negative pressure; whereas, when dogs are made to expire against resistance, only insignificant changes in the intrapleural pressure occur. The pathological changes in the lungs of dogs that expire against resistance are minimal. On the other hand, severe pulmonary

edema at the hilar and basal regions, and peripheral emphysema occur in the lungs of dogs that inspire against resistance.

In patients with bronchial asthma the intrapleural negative pressure may be from 7 to 10 times the normal value. The effect of the increased intrapleural pressure is to exert a sucking action on the capillaries lining the bronchiolar and alveolar walls. The result is exudation of edematous fluid. Barach (15) has demonstrated that there is a definite lowering of the intrapleural negative pressure following the inhalation of a mixture of 80 per cent helium and 20 per cent oxygen. Therefore, the administration of a mixture of helium and oxygen to the patient with intractable asthma is physiologically sound.

Generally speaking there are two types of apparatus for the administration of helium and oxygen. One type is the mask or mouthpiece apparatus of which the recently developed B.L.B. (18) mask is a fine example. This mask may be used for 1 to 3 hour periods of administration. It does not provide a means of rebreathing the helium, to prevent the loss of this valuable gas. The other type of apparatus is the more expensive tent-like equipment which was developed by Barach and his coworkers (15). With this apparatus the helium and oxygen may be administered continuously and the helium can be rebreathed. Another advantage of this more refined equipment is that the helium-oxygen mixture can be given under positive pressure. It is important to add that an ordinary oxygen tent cannot be used for the continuous administration of helium for there are too many opportunities for nitrogen to replace helium even in the best of such tents.

It has been our practice to digitalize patients with intractable asthma. Digitalization should be carried out in 24 to 48 hours. A maintenance dose of digitalis may then be provided until the asthmatic symptoms have subsided.

Physical rest and sleep, if possible, are highly desirable. Unfortunately, the ordinary sedatives employed in treating patients with acute asthma are ineffective. Opiates depress the respiratory center and for this reason alone should not be administered. Maytum (19) first suggested the rectal instillation of equal parts of ether and olive oil. Although there is some doubt as to the safety of rectal ether in such doses as Maytum originally suggested, there is no doubt

that this method of providing rest and relaxation is at times a very beneficial one. The desired effect can frequently be obtained by administering 60 to 120 cc. of such a mixture.

Following the same line of reasoning and with the same results in mind, Fuchs (20) suggested the use of "avertin" (trade name of the Winthrop Chemical Company for tri-brom ethyl alcohol). The "avertin" is now supplied only as "avertin fluid" (tri-brom ethyl alcohol with amylene hydrate). The dose of "avertin fluid," as suggested by Fuchs, is a safe anesthetic dose, according to the report of the Council on Pharmacy and Chemistry in 1937 (21), but factors other than anesthesia alone must be considered for the patient with intractable asthma.

To understand the dangers accompanying the use of "avertin fluid" in intractable asthma, it is necessary to become acquainted first with its potential dangers as an anesthetic. Beecher (22), in an excellent article, enumerated the following causes for the decline in use of "avertin fluid" at the Massachusetts General Hospital: (1) lack of uniform absorption, (2) rectal irritation, (3) depression of the respiratory center with reduction of oxygenating powers of the body, (4) depression of the vasomotor center with fall in the blood pressure, (5) delayed effects such as prolonged unconsciousness which interfere with constant and intelligent nursing, and (6) higher death rate than obtained with any other general anesthetic.

When one considers the patient with intractable asthma, an anoxemic and cyanotic candidate for pulmonary edema and circulatory failure, it then becomes obvious that the risks entailed in employing avertin, outweigh any benefit that might be anticipated. Finally, in taking a firm stand against the advisability of "avertin fluid" in intractable asthma, I can do no better than quote the words of Sir Francis Shipway (21), "The practitioner who is called upon to administer anesthetics on few and irregular occasions, or is but a beginner in this arduous and hazardous specialty will be well advised to leave avertin severely alone."

TREATMENT OF PATIENTS WITH SEVERE CHRONIC BRONCHIAL ASTHMA

There is a large group of patients who, despite thorough allergic survey, irradication of infection, and surgical removal of nasal lesions,

have daily recurring attacks of asthma. To obtain relief these patients resort to frequent daily and nocturnal injections of epinephrine hydrochloride. A method of therapy which will lessen the number of injections and provide the patient with more prolonged relief, attracts the attention of both the patient and the physician.

Because of the known effectiveness of epinephrine hydrochloride in relieving paroxysms of asthma, it is not surprising that the patient and physician should look with enthusiasm upon epinephrine-in-oil. The prolonged effectiveness of epinephrine-in-oil depends upon the fact that the absorption of the epinephrine is mechanically delayed. The diffusion of the oily suspension through the tissues is hindered by the film of oil and the oil, furthermore, restricts the contact of the epinephrine particles with the tissues.

It has been adequately demonstrated (2) that epinephrine-in-oil provides relief from asthmatic symptoms in patients with chronic bronchial asthma for periods of 8 to 16 hours. Patients who had previously been taking frequent injections of epinephrine hydrochloride were able to receive more prolonged relief from fewer injections of epinephrine-in-oil.

One cc. of epinephrine-in-oil is usually a safe initial dose for a patient who is accustomed to frequent injections of epinephrine hydrochloride. Subsequently, the dose may be increased or decreased depending upon the patient's response. One patient may be made comfortable with 0.75 cc., while another may require 2.0 cc. Because so much can be gained by providing uninterrupted rest, it is preferable to administer the initial injection in the evening. Later a morning and even a noon injection may be added if necessary. As symptomatic improvement becomes apparent it is possible to decrease the number of injections and reduce the dose to that amount which will maintain relief from symptoms. If asthma is present at the time when epinephrine-in-oil is administered, an injection of epinephrine hydrochloride beforehand is advisable to provide immediate relief of symptoms. When frequent consecutive administrations of epinephrine-in-oil are to be given it is preferable to make the injections intramuscularly in the deltoid or gluteal muscles.

For the past 13 years the medical literature has contained fairly optimistic reports by Taylor (23), Fink (24), Anderson (25), Cole and

Harper (26), Balyeat and Seyler (27), Mandelbaum (28), and others, on the effectiveness of the intratracheal injection of iodized oils in the treatment of bronchial asthma. To instill the oil two instruments, the bronchoscope and the laryngeal syringe, have been used. The latter instrument has been more commonly employed because it is more adaptable to the average physician's skill. Although there is a group of physicians who claim better results by using the bronchoscope, there are insufficient data to warrant the general acceptance of such an impression.

The scheme of treatment with iodized oil consists of an original instillation of 20 cc. in one side of the lungs, followed by the introduction of the same amount into the other side the ensuing day. An x-ray film should be taken immediately after each injection. If treatment is to follow, 10 cc. of the oil are injected in each side approximately 4 days later and from then on, once each week. After a number of instillations has been given, the interval of treatment may be increased to 2, 3 or 4 weeks according to the requirements of each patient. This treatment may be continued for months or until relief is manifest or failure is evident. The benefit is supposed to be due to the mechanical displacement of mucous by oil, thereby allowing the patient better bronchial drainage.

Recently, an excellent report by Criep and Hampsey (29) has called attention to an increasing skepticism concerning the value of this method of treatment. These authors mailed questionnaires to the members of the two Societies for the Study of Asthma, the American Bronchoscopic Society, and the Trilogical Society. Two hundred and thirty physicians replied. One hundred and sixty-six confessed lack of experience with this form of therapy. The remaining 64 reported on a total of 267 cases of bronchial asthma. Of this group there was absolute failure to obtain relief in 73.4 per cent of the patients, improvement in 24.7 per cent, and a cure in 1.9 per cent. The replies, furthermore, indicated an unusual lack of enthusiasm for this method of treatment. Untoward effects, such as allergic reactions to the iodine and poppyseed oil, circulatory failure, spread of infection, and damage of lung tissue, contributed disadvantages which prevented its whole-hearted acceptance. From such a report, one must conclude that only a small percentage of a well selected group of patients

with severe chronic bronchial asthma can expect temporary benefit from the use of intratracheal injections of iodized oils.

There is a small group of patients with chronic bronchial asthma who fail to obtain relief from any method of medical treatment. In an attempt to offer such patients relief, surgeons have focused their attention upon the surgery of the extrinsic pulmonary nerve supply, and as a result various operative procedures have been advanced. Patients harassed by years of suffering are easy marks for the ambitious investigator and it is, therefore, imperative for physicians to become acquainted with the fundamental principles of these different procedures. Before this is possible, some knowledge of the innervation of the bronchi and lungs by the autonomic nervous system is essential. To understand adequately the innervation of the bronchi and lungs, it is first necessary to become acquainted with the broad principles on which the autonomic nervous system is constructed.

As in the somatic spinal reflex arc, three neurones are involved. There is the afferent neurone which proceeds from an internal organ. Then there is the connector cell which is situated in the gray matter, for example, the lateral horn in the thoracic portion of the cord. The fibers attached to these cells, known as the connecting fibers, join the afferent neurone with the efferent or excitor neurone. The connecting fibers are generally referred to as preganglionic fibers. Lastly there are the excitor cells which for the most part represent ganglia. From the excitor cells, fibers arise which innervate the different organs. These excitor fibers are the efferent or post-ganglionic fibers. The autonomic nervous system may be divided according to the anatomic situation of the connector cells and their fibers. In the brain, these fibers running in their respective cranial nerves, go to make up the cranial autonomic system. In the thoracico-lumbar region, these fibers and their respective excitor neurones go to make up the thoracico-lumbar autonomic system. Likewise in the sacral region the sacral autonomic system is formed. The cranial and sacral autonomic systems are commonly referred to as the parasympathetic system, while the thoracico-lumbar division is frequently called the sympathetic system.

The lungs are innervated by both the parasympathetic and sympathetic systems (Fig. 1). The parasympathetic fibers originating in

the dorsal nucleus of the vagus nerve are carried in the vagus nerve as connector (preganglionic) fibers until they reach the hilus of the lung, where they divide into numerous branches which go to the same and opposite lung. These fibers form a part of the posterior pulmonary plexus on either side and end in ganglia along the main bronchi. From these ganglia excitor (postganglionic) fibers arise which innervate the bronchial musculature. These fibers are known

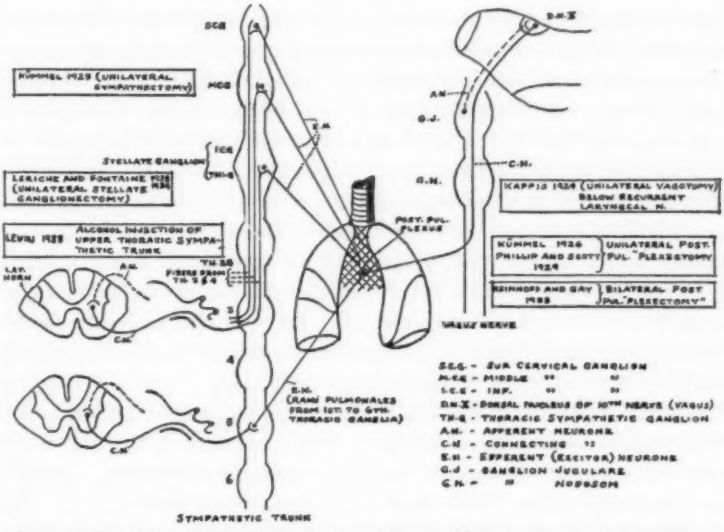
KUMMEL 1929 (UNILATERAL SYMPATHECTOMY)
STELLATE GANGLION
LERICHE AND FONTAINE 1928 (UNILATERAL STELLATE GANGLIONECTOMY)
LEVIN 1929 ALCOHOL INJECTION OF UPPER THORACIC SYMPATHETIC TRUNK
SYMPATHETIC TRUNK
POST. PUL. PLEXUS
VAGUS NERVE
KAPPIS 1924 (UNILATERAL VAGOTOMY) BELOW RECURRENT LARYNGEAL N.
KUMMEL 1926 PHILLIP AND SCOTT 1929 UNILATERAL POST. PUL. PLEXECTOMY
REINHOFF AND GAY 1933 BILATERAL POST. PUL. PLEXECTOMY
S.C.G. - SUP. CERVICAL GANGLION
M.C.G. - MIDDLE " "
I.C.G. - INF. " "
D.N.X - DORSAL NUCLEUS OF 10TH NERVE (VAGUS)
T1-6 - THORACIC SYMPATHETIC GANGLION
A.N. - AFFERENT NEURONS
C.N - CONNECTING "
E.N - EFFERENT (EXCITOR) NEURONS
G.J - GANGLION JUGULARE
G.N. - " NODOSUM

FIG. 1. DIAGRAM OF THE INNERVATION OF THE LUNGS BY THE AUTONOMIC NERVOUS SYSTEM

The names of surgeons and their respective operations are blocked in, at appropriate sites, in the diagram.

physiologically as the bronchoconstrictor fibers. In other words, it has been shown that when the vagus nerve is cut and the distal end stimulated the bronchi of the same, and to a lesser extent the opposite lung, constrict.

The sympathetic system likewise contributes to the make-up of the posterior pulmonary plexus. The connector (preganglionic) fibers which originate in the third and fourth thoracic segments, course

through the anterior roots into their respective thoracic ganglia and then upward to the stellate ganglion. It is likely that fibers, also, go to the middle and superior cervical ganglia. From these ganglia the excitor (postganglionic) fibers arise which, traveling part of the way with the cardiac nerves, finally reach the posterior pulmonary plexus. Also, from the first to sixth thoracic segments connector fibers arise which course to their respective thoracic sympathetic ganglia and then via excitor (postganglionic) fibers, through the rami pulmonales, travel to the posterior pulmonary plexus. There are then two distinct and different sympathetic pathways to the lungs. These sympathetic fibers are known physiologically as the bronchodilator fibers. Undoubtedly afferent and efferent fibers run side by side to and from the lungs in these different sympathetic pathways. The same is so for the parasympathetic tract. There are, also, anastamoses between the two autonomic tracts so that the vagus carries a few of the sympathetic or bronchodilator fibers and the sympathetic carries a few of the parasympathetic or bronchoconstrictor fibers.

With this knowledge of the extrinsic nerve supply to the lungs, it is possible to evaluate more critically the theoretical merits of the various proposed operations on the extrinsic nerve supply to the lungs for the treatment of bronchial asthma (Fig. 1).

Kümmell (30) made the first venture in this field by performing a unilateral cervical sympathectomy. He was aware of the important part played by the vagus but was afraid to sever this nerve. Leriche and Fontaine (31) a little later, and again more recently reported upon the results they had obtained by removing the stellate ganglion on one side. Kappis (32) reported encouraging results obtained by cutting one vagus just below the point of emergence of the recurrent laryngeal nerve. Therefore, equally good results had been reported to follow the interruption of two different pathways, the sympathetic and the parasympathetic, which possess actions antagonistic to each other. An attempt was made to account for these curious results on the basis of a reflex arc, of which the sympathetic fibers made up the afferent and the vagus fibers the efferent neurones. Kümmell (33), in in 1926, sectioned the posterior pulmonary plexus on one side, and thereby removed all the extrinsic nerve supply to one lung. Phillips and Scott (34) reviewed the literature in 1929, and endorsed Kümmell's operation and reported a case of their own. Levin (35),

in 1935, suggested alcoholic injection of the upper thoracic sympathetic ganglia. Recently, Reinhoff and Gay (36) reported encouraging results following bilateral resection of the posterior pulmonary plexus.

This small group of surgeons represent only the pioneers and the originators of their respective operative procedures. It is necessary to call attention to the fact, that despite the type of operation, all procedures have been reported to have brought about some brilliant results. Furthermore, one need only read over carefully the numerous publications to become aware of the large number of complete failures. It is impossible to advocate one operation by relying entirely upon reported results. If one were to approach the evaluation of these various procedures on purely theoretical grounds, based upon our present day knowledge of the anatomy and physiology of the autonomic nerve supply to the lungs, one would be forced to choose the operation suggested by Reinhoff and Gay. It is only logical to believe that if interruption of either the sympathetic or vagus is sometimes effective, sometimes ineffective, sometimes partially beneficial, that a combination of these procedures might offer even better results. Furthermore, bilateral interruption of these tracts should offer greater improvement than unilateral interruption. Unfortunately, there is more than theory to consider. The operation as suggested by Reinhoff and Gay is a drastic procedure which should be attempted only by a skilled surgeon possessing a profound knowledge of operative details. The physician must remain ever critical; he must realize that established anatomical changes are not likely to be erased by lung denervation; and he must bear in mind that bronchospasm may theoretically occur, even after section of both the posterior pulmonary plexuses, by stimulation of the ganglia in the bronchi through an intrapulmonary reflex arc.

SUMMARY

A discussion of the medical and surgical treatment of patients with severe bronchial asthma is simplified by dividing the patients into 3 groups, namely, (1) patients with severe acute bronchial asthma, (2) patients with intractable asthma ("status asthmaticus"), and (3) patients with severe chronic bronchial asthma.

Patients with severe acute asthma should first receive 0.3 to 0.5 cc.

of epinephrine hydrochloride (1:1000). Such a dose should be repeated every 15 minutes until 4 doses have been administered or until relief is apparent. To prevent recurrence of asthma, an intramuscular injection of 0.75 to 1.0 cc. of epinephrine-in-oil, should follow. One of the barbituric acid derivatives may be employed as a sedative. An injection of 0.3 to 0.5 cc. of Schlesinger's solution is permissible if the patient is badly in need of rest. Evidence is advanced in favor of the use of epinephrine therapy for hypertensive patients with severe asthma.

Patients with intractable asthma are acute medical emergencies. These patients fail to obtain relief from the usual epinephrine preparations, but they have been wrongly considered to be "epinephrine-fast." The intravenous injection of 100 cc. of 50 per cent sucrose to which has been added 0.5 cc. of epinephrine hydrochloride (1:1000), or the injection of 0.48 gm. of aminophyllin diluted in 10 cc. of normal salt solution, may give temporary relief. The continuous or intermittent inhalation of an atmosphere of 100 per cent oxygen, or of 80 per cent helium and 20 per cent oxygen is a physiologically sound therapeutic measure. To improve the cardiac action, digitalization should be carried out in 24 to 48 hours. A maintenance dose of digitalis should then be provided until there is freedom from asthmatic symptoms. Sixty to 120 cc. of a mixture of equal parts of ether and olive oil may be instilled rectally to induce sleep. The use of avertin, to induce sleep, is dangerous. Opiates, because they depress the respiratory center, should not be given.

Patients with severe chronic bronchial asthma require frequent daily and nocturnal injections of epinephrine hydrochloride (1:1000). The use of intramuscular injections of epinephrine-in-oil permits fewer injections and is followed by longer periods free from symptoms. The value of intratracheal injections of iodized oils is generally over-rated. Following a discussion of the autonomic innervation of the lungs, the merits of such operations as unilateral sympathectomy, unilateral stellate ganglionectomy, unilateral vagotomy, and unilateral and bilateral posterior pulmonary "plexectomy," are considered.

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THE ACTION OF QUININE METHOCHLORIDE ON NEUROMUSCULAR TRANSMISSION

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In a previous paper (Harvey, 1939), it was shown that quinine has, among many actions on muscle, a relatively weak one of the type of the action of curarine. It is known that quaternary ammonium compounds formed by the addition of alkyl radicles to the nitrogen atom of the quinoline ring have a curare-like action on the neuromuscular junction of the frog. (Santesson and Koraen, 1900). It was not unlikely, therefore, that the curariform action of quinine would be greatly increased by the formation from it of quinine methochloride. This drug, prepared for me by Dr. H. King, has been found to have effects upon the neuromuscular junction similar to those of curarine chloride (King, 1935).

METHODS

The effect of the drug upon the muscle response to nerve stimulation was studied in cats, decerebrated under preliminary ether, and prepared for recording from the tibialis anterior muscle according to the technique of Brown (1938). The methods for mechanical recording, for stimulation, and for the recording of muscle action potentials were essentially the same as those previously described from this laboratory (Brown and Euler, 1938; Brown and Harvey, 1939). The action potentials of the phrenic nerve were led off, by means of electrodes of cotton wool soaked in Locke's solution, to the input of a 5 stage condenser-coupled amplifier, which was connected to a loud speaker and to a Matthews' oscillograph. For the preparation of the superior cervical ganglion for perfusion the tehnique described by MacIntosh (1938) was used. The blood pressure of the cat, under chloralose, was used for measuring the output of acetylcholine in the perfusion fluid.

The quinine methochloride was dissolved in saline solution (0.9 p.c.) acidified to pH 4. Control injections of the acid saline caused no alteration in the muscle

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responses. Eserine and curarine were prepared in a similar manner. Acetylcholine solution in acid saline at pH 4 was always prepared just before use.

RESULTS

Effect of Oral Administration: When 150 to 200 mg. of quinine methochloride per kg. is given by mouth, a sequence of events follows which is similar to that described by West (1936) as following an injection of curarine in human beings. First, the muscles of the jaw and the extrinsic ocular muscles become weak. This effect is followed in turn by loss of movement in the limbs, disappearance of the tendon reflexes, and, finally, paralysis of the diaphragm. The heart continues to beat vigorously, but the animal dies from asphyxia unless artificial respiration is instituted. This could be applied without injury, by passing a catheter of suitable size through the glottis into the trachea, and connecting to a respiration pump. Just before the paralysis is complete there may be a few twitchings of the muscles, especially those of the trunk. When the effects of the drug begin to wear off, spontaneous respiratory movements begin, later the reflexes return, and 12 hours later the animal is fully recovered. The substance has no apparent secondary actions, and the animal, recovered from its paralytic effect, is apparently restored to completely normal health.

Effects When Otherwise Administered: When the methochloride is given by slow intravenous infusion to a decerebrate cat, a complete paralysis appears suddenly when about 15 mg. have been given, and lasts for about one hour. Subcutaneously, a total of 80 mg. per kg., administered in divided doses over a period of two hours, results in the gradual development of paralysis, which persists for 3 to 4 hours, partial paresis continuing for 12 to 14 hours. These doses compare with that of 150 to 200 mgm. per kg. required, as above described, to produce a comparable paralysis with oral administration.

The Action upon Neuromuscular Transmission: The effects of quinine methochloride upon the mammalian nerve-muscle preparations are almost identical with those of curarine, though requiring a higher level of dosage for their production. After an intravenous dose of 10 to 15 mg. per kg. the responses of the muscle to maximal motor nerve timuli are quickly abolished (Fig. 1), while the twitch evoked by direct stimulation of the muscle remains unaltered (Fig. 2). Even

before the twitch tension is appreciably lowered, the response to repetitive stimulation of the nerve is diminished, and the tension of the muscle cannot be maintained. The decurarizing effect of a motor

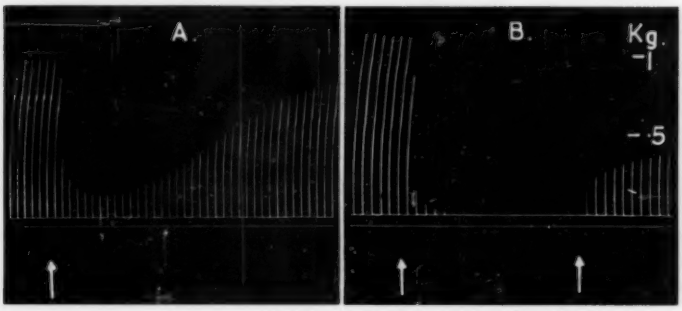

FIG. 1. Cat, decerebrate, 3.1 kg. Maximal motor nerve stimuli of tibialis anterior every 10 sec. A. At arrow 5 mg. quinine methochloride intravenously. B. At arrow 1, 15 mg. quinine methochloride intravenously. Arrow 2 maximal motor nerve tetanus at 50 per sec. for 5 sec.

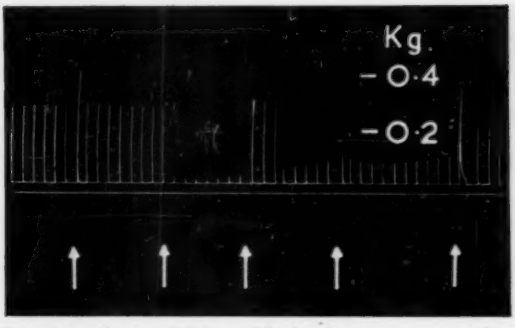

FIG. 2. Cat, decerebrate, 2.2 kg. Maximal motor nerve stimuli of tibialis anterior every 10 sec. At arrows 1 and 4, inject 5 μg. aetylcholine intra-arterially; arrow 2, quinine methochloride 10 mg. intravenously; arrow 3, three maximal direct stimuli; arrow 5, maximal motor nerve tetanus at 50 per sec. for 5 sec.

nerve tetanus (Boyd, 1932), can be easily demonstrated (Fig. 1, B). This action occurs, even though there is no visible mechanical response of the muscle accompanying the repetitive stimulation of the nerve.

Also, as in the case of curarine, the twitch-like response of the muscle evoked by the close arterial injection of acetylcholine is abolished before the responses to nerve stimulation disappear (cf. Brown, Dale and Feldberg, 1936). Figure 2 shows the reduction in the size of the response to injected acetylcholine after the administration of a small dose of quinine methochloride. The potentiating action of eserine upon the responses of the muscle to maximal motor nerve stimuli is counteracted by doses of the drug which do not entirely block transmission. Once full neuromuscular block has been produced, eserine

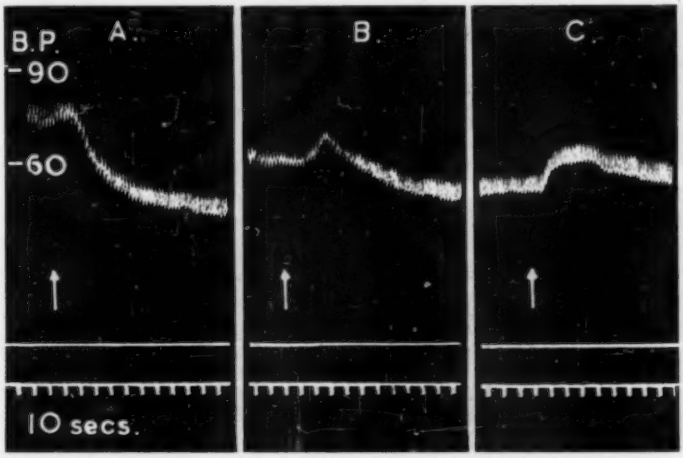

FIG. 3. Cat, decerebrate, 2.5 kg. Carotid artery, blood pressure. Intravenous injections of quinine methochloride. At A, 5 mg.; B, 25 mg. and C, 100 mg.

is able to relieve it, although the doses required for this action are large. When the animal is fully paralysed with curarine itself, and the muscle is stimulated directly, doses of quinine methochloride sufficient in themselves to block neuromuscular transmission cause no appreciable alteration of the contraction in response to single shocks, or to tetanic stimulation.

The Effect on Blood Pressure: The effects of quinine methochloride upon the blood pressure are similar to those of quinine. (Nelson, 1927; Niederehe, 1918). When injections are made intravenously at a

rather rapid rate, the first dose causes a moderate fall of blood pressure which may be of fairly long duration (Fig. 3). With the second, and subsequent doses a rise of blood pressure usually accompanies the administration of the drug. However, the deleterious effects upon the

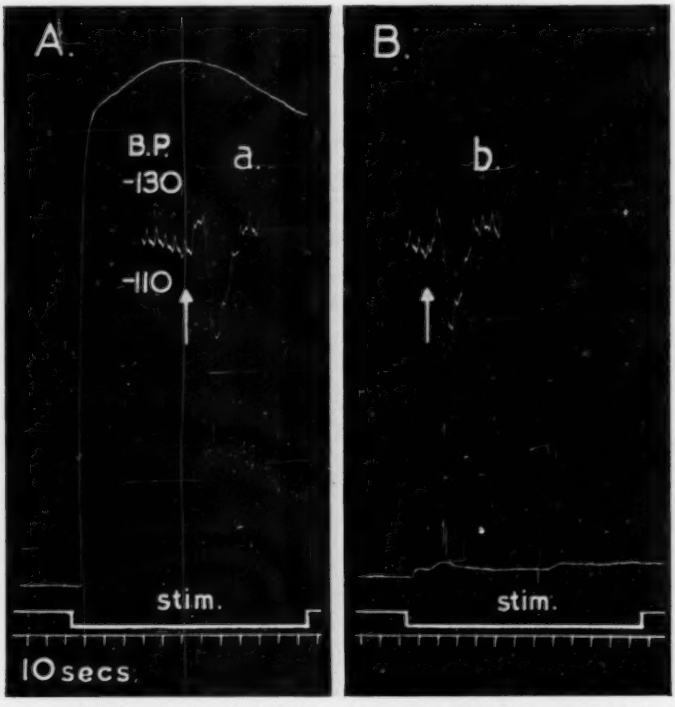

FIG. 4. Ganglion perfused with Locke's solution containing eserine, 2 in 10^6. Contractions of nictitating membrane in response to preganglionic stimulation at 10 per sec. Between A and B quinine methochloride 2 mg. in 0.5 cc. added to perfusion. a. and b., effects on cat's blood pressure (B.P.) of 1 cc. of fluid collected during periods A and B. Blood pressure response abolished by atropine.

circulation cannot be very severe, since animals survive doses more than twice those necessary to produce complete paralysis, provided that artificial respiration is maintained.

The Effect upon the Respiratory Centre: In order to determine the

state of the medullary centres during complete peripheral paralysis with quinine methochloride, the phrenic nerve was dissected in the neck of a decerebrate cat, and the central end arranged for the recording of its action potentials. The animal was placed on artificial respiration of such a volume that some respiratory movements continued. A full paralysing dose of the drug was then given. The discharge from the central end of the phrenic nerve was unchanged. Stopping the artificial respiration at this stage led to the characteristic increase in

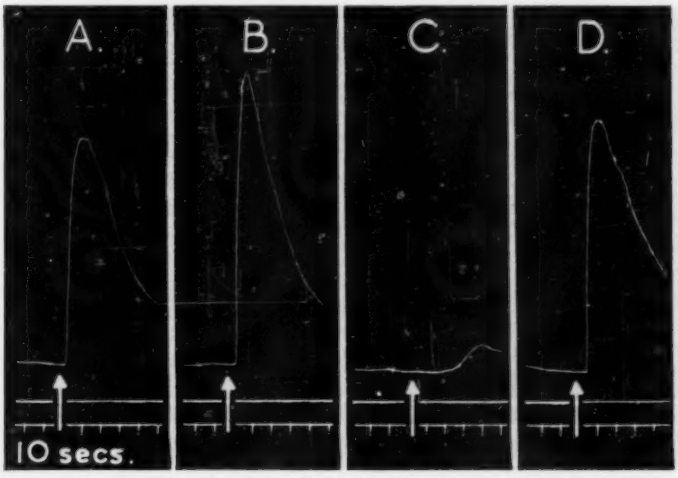

FIG. 5. Same experiment as Fig. 4. At A and C injection of 2 μg. of acetylcholine. At B and D injection of 2 mg. of KCl. Between B and C, 2 mg. of quinine methochloride in 0.5 cc.

phrenic activity, as soon as central asphyxia developed. Quinine methochloride has, therefore, no obvious central action.

The Effect upon Ganglionic Transmission: Quinine methochloride added to the perfusion fluid (2 to 4 mg. in 0.5 cc.) depressed ganglionic transmission as shown by failure of the nictitating membrane to contract (Fig. 4). However, just as with curarine, there is still the usual output of acetylcholine as shown by testing the venous effluent from the ganglion on the blood pressure of the cat. The similarity to curarine is made more complete by the fact that the response of the gan-

glion cells to injected acetylcholine is abolished, while that to potassium chloride remains essentially the same (Fig. 5). This differential paralysis of the superior cervical ganglion following the administration of curarine was demonstrated by Brown and Feldberg (1935).

DISCUSSION

In the main features of its action on the mammalian nerve-muscle preparation, quinine methochloride resembles curarine, blocking neuromuscular transmission and leaving the response of the muscle to direct stimulation unimpaired. Like curarine it causes a depression of the response of the motor endplate and the ganglion cell to nerve impulses and to acetylcholine, but does not interfere with the normal liberation of acetylcholine from the preganglionic nerve endings. At the same time it leaves relatively unaffected the normal discharges from the respiratory centre as shown by the persistence of spontaneous action potentials in the phrenic nerve after complete peripheral paralysis of transmission. It may be noted that this method of approach provides an easy way of distinguishing between the central and peripheral action of a drug of this type.

Quinine has been shown to have a curariform action (Harvey, 1939), and this effect is probably due to the presence of the tertiary nitrogen group of the quinuclidine nucleus. By the addition of a methyl group this is converted into the quaternary nitrogen compound used in the present experiments, which exhibits this curare-like action to a much greater degree. The most characteristic action of quinine on muscle, apart from its weak curare-like effect, is its property of increasing the tension of single muscle twitches excited directly or through the motor nerve. The introduction of the methyl group has clearly not only intensified the curariform action, but has to a very large extent suppressed the other effects of quinine; as considerable doses of the methochloride do not alter the tension of the directly excited twitch.

Curarine has been found to have certain uses in clinical medicine. West (1936) has tried it in the treatment of cases of general tetanus, and it has been found helpful in certain spastic conditions (Burman, 1939). Pure preparations of curarine are difficult to obtain, however, and are not effective when given by mouth. Quinine methochloride produces its action when administered orally, and it is easy to prepare

in a state of purity, being a chemical compound of known structure, so that its dosage can accordingly be determined with accuracy, if it should be desired to produce a curare-effect.

SUMMARY

1. Quinine methochloride, formed by the addition of a methyl group to the quinuclidine nucleus of the quinine molecule, has a strong curare-like action.

2. It blocks transmission at the neuromuscular junction, and at the synapses in the superior cervical ganglion without interfering with the normal discharges from the respiratory centre in the medulla.

3. Its action is like that of curarine in causing a depression of the response of the ganglion cell to nerve impulses and to acetylcholine, without interfering with the normal liberation of acetylcholine from the preganglionic nerve endings.

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ON THE CHROMOSOMAL NATURE OF NUCLEOLI[1]

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The significance of nucleoli was unknown until it was shown that they are associated with particular regions of particular chromosomes in Vicia by Heitz (1931) (also in Drosophila by Kaufman (1934), in Zea by McClintock (1934), in Amblystoma by Dearing (1934), in opalinids by Chen (1936), and in Chironomus by Heitz and Bauer (1933) and Paulson and Metz (1938)[2]). Are nucleoli likewise associated in some manner with chromosomes in the cells of higher vertebrates?

A critical examination of our motion pictures of normal dividing rat fibroblasts in tissue cultures seems to show that here the larger "nucleoli" split up into chromosomes or parts of chromosomes in prophase and that large new "nucleoli" are formed by the clumping of chromosome material in the daughter nuclei.

NUCLEOLI IN PROPHAS

The resting nuclei of most normal living fibroblasts as seen in young cultures have a homogenous nucleoplasm and one to several masses of various sizes ordinarily referred to and herein designated as nucleoli. The nuclei of rat fibroblasts that have been actively dividing for a few weeks *in vitro* usually contain considerable nucleolar material partly in the form of granules which may give a somewhat coarsely granular appearance to the nucleus.

One of the early indications of the beginning of prophase is the appearance of granules (chromosomes) in the nucleoplasm. They slowly increase in number and size and become thickly scattered throughout the nucleus, but more or less separated from one another by nucleoplasm. The nucleoli seem to remain unchanged during

[1] Aided by a grant from the International Cancer Research Foundation.

[2] *Jour. Morph.* **63**: 363–395. References to the preceding authors.

this process, but owing to the now granular condition of the nucleus the larger ones become less conspicuous and the smaller ones are not to be distinguished from the chromosomes.

Practically nothing is known about the reaction which results in the chromosomes becoming visible. It is probably some sort of a condensation and increased gelation of the chromatin material of the swollen chromosomes, which are supposed up to this time to fill the nucleus. The immobility of the visible chromosomes would seem to indicate that the nucleoplasm between them is a gel.

SPLITTING OF NUCLEOLI

At about the time the chromosomes attain their maximum visibility and number, each of the larger nucleolar masses splits up into two or more bodies of approximately the same size and appearance as the chromosomes. This takes only about 2 or 3 minutes. Most cells tend to become spherical at this stage and the process is often obscured but every cell that remained somewhat spread out at the time the motion pictures were made showed this splitting of the larger nucleoli. The smaller nucleoli seem to remain as chromosomes. These chromosome-like bodies can be followed until chromosomal movements begin. After that the confusion is too great to follow them and thus there is no proof of their actual persistence through the rest of division.

It has not been possible to determine from the motion pictures as to whether the nucleolar chromosome material formed whole chromosomes or only parts of some of those that came into view at this time.

MOVEMENTS OF CHROMOSOMES

During the early prophase and until the nucleoli have split into chromosomes, the nucleoli and chromosomes remain immobile. The cytoplasmic inclusions, fat globules, mitochrondria and neutral red granules, on the other hand, always show considerable motion. Three to four minutes after the nucleoli become split up, all chromosomes including those from nucleoli begin to show definite individual movements. Soon after this, mass movements begin and continue until chromosomes are arranged on the metaphase plate. The movements of the chromosomes are in reality so slow that it is almost impossible

to follow them with the eye; in the motion pictures, where motion is speeded up 24 to 80 times they are, however, quite evident and conspicuous, and give the appearance of an agitation.

The movements continue in metaphase and become a slow oscillation back and forth toward the poles. All oscillation ceases after the chromosomes split and start moving to the poles of the spindle.

A few cells which had two large nucleoli showed the movements of chromosomes grouped into two independent fields about the disintegrated nucleoli. The fields may remain distinct until the chromosomes become arranged in the metaphase plate. This suggests that there is a definite grouping of chromosomes with reference to the nucleoli.

Any explanation of the movements is a mere guess. The motions, as already noted, are slow and thus not Brownian. About the time of the beginning of these movements the nuclear membrane disappears. The exact time relationship has not been determined and there is no special indication that the two are related. The movements are probably the beginning of the process which leads to the arrangement of the chromosomes on the metaphase plate. How is this accomplished? The process takes several minutes. As one watches it in the motion pictures one is not quite certain as to the exact moment of the beginning of metaphase. There is a sort of premetaphase stage before the chromosomes are neatly arranged in the median plate, during which it is difficult to decide whether the cell is in late prophase or early metaphase. I have rather doubted the existence of spindle fibers, never having seen them in healthy living cells, but, now, something of the sort seems to offer an explanation of the movements of the chromosomes during this premetaphase and their oscillation during metaphase. If we look upon the spindle fibers as invisible gelated bands which exert contractile tension they would pull on the chromosomes. Variation of the tension resulting from changes of their viscosity would result in pulls first toward one pole and then toward the other until finally they are pulled into the metaphase plate, where they oscillate back and forth until they (the chromosomes) split and begin moving toward the poles.

FORMATION OF DAUGHTER NUCLEI AND THEIR NUCLEOLI

As the chromosomes pass to the poles during anaphase they come closer and closer together and form at each pole a small

irregular compact mass. The borders of the mass are usually irregular and jagged, especially on the abpolar aspect where some of the chromosomes project.

After the daughter nuclei reach the poles they begin to swell. Small clear areas appear, enlarge and expand the mass. A nuclear membrane forms. As the nucleus expands, the visible chromosomal material becomes reduced in the course of 5 to 10 minutes to scattered granules in a gradually increasing nucleoplasm. Some of them become closely attached to one another to form the larger nucleoli; others persist as smaller nucleoli but most of them disappear in the course of an hour or two.

During certain periods of the growth, daughter nuclei resemble prophase ones. They are smaller but otherwise difficult to distinguish from prophase ones when searching in the motion picture setup for suitable cells to photograph. Part of the growth of daughter nuclei is a sort of reverse prophase up to the time of the movements of the chromosomes. Prophase starts with clear nucleoplasm and nucleoli; chromosomes appear and nearly fill the nucleoplasm; then the nucleoli split up into similar bodies. The compact daughter nuclei loosen up into granules; some of the latter clump into nucleoli; others disappear and leave a clear nucleoplasm with one to several nucleoli, the resting cell condition. During this part of prophase and during the growth of the daughter nucleoli, the chromosomes are immobile in contrast to the agitation of cytoplasmic inclusions such as fat globules, mitochondria and neutral red granules.

Here again any explanation of the processes underlying the disappearance of most of the chromosomes, the simultaneous enlargement of the nucleus, the appearance of the nuclear membrane and the persistence in visible form of some chromosomes or parts of them to form nucleoli is mere guesswork, but guesses must be made until one is found that can be substantiated by experimental procedures. The obvious explanation of the disappearance of the chromosomes is that each one swells up, that it takes up fluid and loses its high index of refraction. This naturally results in the enlargement of the nucleus. The nuclear membrane is probably something more than an interface membrane. I have no explanation as to why certain chromosomes or parts of certain ones fail to disappear and remain visible as nucleoli. It is not known if the same ones take part in

the formation of the nucleoli, generation after generation, as in Vicia, Zea, Drosophila, Chironomus, Amblystoma and opalinids. The clumping is probably somewhat accidental because of the variations in number and size and location of the nucleoli in different cells.

NUCLEOLI OF RESTING CELLS

In the course of 2 or 3 hours, daughter nuclei become approximately resting cell size and the nucleoli become fixed. They remain constant as regards number, size and location for many hours, probably until the next mitosis, if the cells remain in good condition. I have followed for 24 hours a few adult rat fibroblasts and for a few hours many others without being able to discern any change in the nucleolar patterns. Each individual cell has its own peculiar nucleolar pattern, no two of many cells examined have been exactly alike in this regard. The nucleolar aggregates vary in number, in size and in location. Thus there are almost an infinite number of possible combinations. A number of pairs of daughter cells have been examined. No two were alike. A few mother and daughter cells were followed; they all had unlike nucleolar patterns.

NUCLEOLI OF MALIGNANT CELLS

It is now quite well known from the observations of MacCarty and his co-workers, Lewis and Lewis and others that the nucleoli of malignant cells average larger than those of normal ones of the same type. Not only are they larger but the nucleus often contains in addition granular material, sometimes in considerable amounts. It has been noted above that normal fibroblasts which have been actively dividing for some time in cultures also contain more than the usual amount of nucleolar material, some of it in the form of granules. Since the normal nucleoli seem to be chromosomal in origin it seems quite probable that the nucleoli and nuclear granules of malignant cells are also chromosomal in nature and that during the growth of the daughter nuclei the chromosomes do not disappear as completely as in normal cells.

CONCLUSION

Nucleoli consist of chromosome material.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Beesly and Johnston's Manual of Surgical Anatomy. Revised by JOHN BRUCE AND ROBERT WALMSLEY. 5th edition. 733 pp. Illus. $6.50. *Oxford University Press, London, New York, etc., 1939.*

The fact that this is the fifth edition since 1916, is indisputable evidence that this is a useful publication. It has been written and revised by men trained in Edinburgh where, it is the reviewers' impression, anatomy is more emphasized, better taught and better understood than in the other medical centers of the world.

The book itself is just what the title implies: a manual of Surgical Anatomy. As such it is written in a concise, terse and positive fashion, with little space given to mentioning possible conflicting opinions. The clinical and practical applications of these anatomical facts are included in a surprisingly large number of instances.

The illustrations are relatively infrequent but, where present, adequately reveal the anatomy or point under discussion.

The physical dimensions of the volume are reduced to those usually found in manuals as compared to the larger size of textbooks. This of necessity, to a certain degree, crowds both the text and the illustrations.

As a manual of surgical anatomy it is excellent, but it will probably be more popularly received by senior students and graduates of surgery in the British Isles than in this country where the larger and more profusely illustrated textbooks of Surgical Anatomy are more generally used.

R. T. S.

The Clinical and Experimental Use of Sulfanilamide, Sulfapyridine and Allied Compounds. By PERRIN H. LONG AND ELEANOR A. BLISS. 319 pp. $3.50. *The Macmillan Company, New York, 1939.*

Dr. Long and Dr. Bliss have accomplished what must have been a mammoth task in the preparation of this excellent monograph; and what is more, though it must have been written with remarkable expedition, it does not produce the impression of a hurried compilation. As the authors state in their preface, the six years that have elapsed "since Foerster reported upon the use of Prontosil in the treatment of a child ill with staphylococcal sepsis" have been "called epochal in the history of medicine". In this short period an immense amount of work has been done with sulfanilamide and its derivatives. Chemical, pharmacological

and biological experimentation; clinical bacteriological and therapeutic observations have been published in unprecedented numbers and in bewildering profusion. An intimate knowledge of the entire situation and a wide personal experience have enabled the authors to present the problem of the chemotherapy of sulfanilamide and its related compounds in an unusually orderly manner and in clear perspective.

The problem is introduced from its historical aspects. This forms the first and highly interesting chapter. In the second chapter there follows a full account of the results obtained from the chemotherapy of experimental bacterial infections by these drugs. The fourth chapter is devoted to a discussion of the mode of action of sulfanilamide and its derivatives, after which there follows the important account of the clinical use of sulfanilamide. This chapter starts with a consideration of the general principles that must be followed when sulfanilamide is employed as a specific chemotherapeutic agent, and appended to this are directions for the auxillary treatment of patients receiving this drug, a matter which is by no means insignificant. The major portion of this chapter, however, is natually occupied with descriptions of the results obtained in the treatment of streptococcal, meningococcal and gonococcal infections by sulfanilamide, though important sections deal with the treatment of infections of the urinary tract and infections by Cl. Welchii—the gas bacillus. In addition, sufficient attention is also given to a considerable number of infections in which the results have been equivocal or unsatisfactory. Then follows in the sixth chapter on account of the clinical use of sulfapyridine, the sulfanilyl sulfanilamides and benzylsulfanilamide. One wishes that the most recent evidence for the value of sulfapyridine in the treatment of pneumococcus pneumonia might have been in print when the book was written, but even so the few pages containing all the information available at that time are prophetic of what has followed. In the seventh chapter the authors discuss the clinical use of prontosil and neoprontosil and in the last chapter describe at length the toxic manifestations of sulfanilamide and its derivatives.

The book is a mine of information covering all possible phases of the subject. The valuable bibliographies at the conclusion of each chapter often run into hundreds of references. But in spite of the inclusion of this prodigious amount of literature, the book is far more than a review, for the subject is developed in a critical manner and is given an individuality through the constant use of knowledge acquired through personal experimentation and clinical observation. The monograph forms an essential reference book to all who work experimentally with these chemicals as well as to the practicing physician who today should have intimate knowledge concerning the therapeutic action of these drugs.

Classic Descriptions of Disease, with Biographical Sketches of the Authors. By RALPH H. MAJOR. 2nd ed. 727 pp. Illus. $5.50. *Charles C. Thomas, Springfield, Ill., 1939.*

It would be difficult to praise this book too highly or to recommend it too vigorously. The writer has drawn his material from original descriptions of im-

portant diseases, selecting from each the material pertinent to the original contribution contained therein. In a surprising number of instances it is shown that the physician whose name has been commonly associated with a great discovery was, in fact, not the first to report the matter credited to him. Thus, heart-block with convulsions, commonly known as the "Adams-Stokes" syndrome, was probably reported several times prior to the communications of these two physicians. The second of such early reports was an excellent description of heart-block in the "De Sedibus—", by Morgagni, published before either Adams or Stokes was born. Laënnec will not lose in stature if it is pointed out that atrophic cirrhosis was described very adequately by John Brown almost exactly a century before Laënnec's first breath at Quimper. It is of no moment to argue the priority of Graves *versus* Basedow, since Parry gave a good picture of exophthalmic goiter before either of these favored candidates for historical honors.

The modern clinician will lose nothing by perusing the meticulous descriptions of the ancients: for example, two of the three earliest accounts of exophthalmic goiter record rheumatic fever and *angina tonsillaris* in the background of their patients—a feature which has interested the reviewer, but which has been largely neglected in the intervening years.

Dr. Major is to be congratulated on bringing Dr. James Hope into his historical Hall of Fame and giving him credit for being, as he was, the great coördinator of cardiology. For it was Hope who brought order out of the chaos, concerning this subject, in which the great clinicians of the early nineteenth century, including Laënnec himself, were floundering. Hope escaped the attention of Garrison, and is not well known even at his own hospital.

In addition to the historical descriptions, Dr. Major has included biographic sketches of admirable literary quality, appropriate brevity and well considered content. Mr. Thomas has manufactured a splendid book. The proof-reading and the English construction gave the reviewer not the slightest cause for criticism. In brief, the book is a contribution to medical annals which the American physician may place among the classic works of this country.

J. T. K.

Physiological Group Differentiation in Lymnaea Columella. By JOSHUA L. BAILY, JR. (Am. Jour. of Hyg. Monographic Series, No. 14, 1939). 133 pp. $1.00. *The Johns Hopkins Press, Baltimore, 1939.*

The investigation reported in this monograph is concerned with the question of race differentiation, but it differs fundamentally from the classical study in this field which deals almost entirely with structural characteristics. This study examines the possibility of physiological differentiation of races which are structurally indistinguishable. The particular traits analyzed are, rate of growth, fecundity, and duration of life.

The evidence presented is for the pond snail, *Lymnaea Columella*, taken from two different geographical localities. Morphologically the snails from the two environments are indistinguishable, but their progeny, under laboratory condi-

tions show marked physiological differences. The physiological ages of the two groups appear to be conspicuously different since there is a decided contrast in the calendar ages at which homologous events take place. This is true of age of first oviposition, average age at which eggs are laid, age of greatest fecundity, age of maximum growth rate, and age at death. There are a number of other striking differences such as in size of infant death rate, span of life, and maximum size attained. Not only do the central values of the two groups differ as to these traits, but the variation of the individual members of the groups show characteristic differences.

This study has very significant implications. The description of various biological populations accumulated in numerous investigations has been concentrated much more intensively on morphological than on physiological traits. For only a very few biological forms do we know the pattern of such a fundamental trait as longevity. The evidence presented here that different strains may be functionally distinct although structurally inseparable, emphasizes the limitation of purely morphological description and the need for a wider knowledge of the physiological patterns of biological populations.

M. M.

Heart Patients. Their Study and Care. By S. CALVIN SMITH. 166 pp. $2.00. *Lea & Febiger, Philadelphia, 1939.*

The intention of the author of this small volume is to present a summary of facts about the heart that will be of particular use to the general practitioner. Through the use of aphorisms and homely expressions he has made an interesting attempt toward his goal. It is unfortunate that the meat of the subject matter is not better, for there is a human touch about the book which indicates that the writer has drawn from his practice a considerable degree of wisdom. The book, however, has definite defects, in the opinion of the reviewer, which make it impossible to recommend as a modern text on cardiac disorders.

J. T. K.

The Evolution and Organization of the University Clinic. By SIMON FLEXNER. 41 pp. $1.25 (Paper bound). *Oxford University Press, New York, London, etc., 1939.*

During Dr. Flexner's sojourn at Oxford as Eastman Visiting Professor, the University was in the process of establishing several new departments of the Medical School in which medicine and surgery are to attain a university status. Dr. Flexner was therefore asked to give two lectures on the significance of this development. These lectures now appear in printed form, as a pamphlet.

At the outset three requirements are mentioned as involved in placing these broad subjects on a university basis; laboratories for scientific research of fundamental importance, clinical professors as leaders in these laboratories, and the provision of time for the professor sufficient for scientific investigation and teaching. Dr. Flexner considers that the key to the achievement of these conditions

lies in the laboratories. He therefore devotes his first lecture to a review of some of the outstanding men and their contributions during the last one hundred years, who have given to medicine its present scientific character. Although this account is somewhat rambling and informal, it shows clearly the main sources from which the spirit of investigation has watered the fields of medicine, until a vigorous growth has ultimately been developed, not only in the biological sciences pertaining to man, but also in the study of disease, its prevention and treatment. The dominant part played by the pioneering scientists of Germany is emphasized. The second lecture deals more specifically with clinical medicine and gives some interesting examples of fundamental contributions that have come from workers attached to medical clinics, indicating the desirability of thorough training in chemistry, physics and biology for the investigation of clinical problems.

It seems clear that Dr. Flexner has emphasized certain phases of organization of the medical clinic which, although generally recognized in this country, require further development in English medicine. He gives an account of the steps which influenced the Johns Hopkins University to place the clinical departments of its medical school on a university basis, and also recalls the demonstration of the ideas of Ludwig and of Welch by the Hospital of The Rockefeller Institute in the strengthening of the scientific background of medicine in this country.

No attempt is made to give a full account of the organization of the University clinic with its complexities of teaching and of the care of patients. Nor are there any details as to how laboratories should be organized as Dr. Flexner fully realizes the necessity of the shifting of interests and emphasis, determined by talents and opportunities as well as by the ever-forward march of medical science. New problems, new techniques and new interests are arising so constantly in medicine that rigid organization of scientific programs and facilities is harmful to the medical clinic as far as its scientific life is concerned. It is the evolution of this phase of the University clinic to which Dr. Flexner directs attention in these lectures in an interesting and stimulating manner.

G. C. R.

Clinical Diagnosis by Laboratory Methods. By JAMES CAMPBELL TODD AND ARTHUR HAWLEY SANFORD. 9th ed. revised. 841 pp. Illus. $6.00. *W. B. Saunders Company, Philadelphia & London, 1939.*

In the new edition of this well known and deservedly popular book, improvements have been made in its weakest chapter, that on the blood, and the material on serodiagnostic tests for syphilis has been completely revised. Other minor changes have also been made.

M. M. W.

BIOGRAPHY OF DR. HARVEY CUSHING

Mrs. Cushing has requested me to prepare a biography of her husband and I should be most grateful to anyone who wishes to make letters, anecdotes or other memorabilia available.

Copies of all letters, no matter how brief, are desired, and if dates are omitted it is hoped that, when possible, these may be supplied (e.g., from the postmark). If original letters or other documents are submitted, they will be copied and returned promptly.

A new Medical Library building is being erected at the Yale University School of Medicine in which Dr. Cushing's library and collections, including his letters, diaries and manuscripts will be placed. Any of his friends who wish, now or later, to present correspondence, photographs or other memorabilia for permanent preservation among the Cushing papers will receive the appreciative thanks of the University.

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THE EFFECT OF STIMULATION ON THE FAT AND CARBOHYDRATE CONTENT OF THE GASTROCNEMIUS MUSCLE IN THE PHLORIZINIZED RAT[1]

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A large amount of evidence supporting the idea that oxidation of fat can supply the energy for muscular contraction has accumulated during recent years. In this paper the experimental data will be reviewed; additional material will be presented to show that fat is not oxidized by mammalian muscles directly; and suggestions will be made as to possible ways in which fat may be utilized indirectly during muscular work.

Circumstantial evidence for fat utilization has been obtained from numerous sources. In the first place, it is common experience that exercise decreases the amount of fat in the body. The fat which disappears must be used either directly or indirectly for energy production. Experimentally, however, only a few studies have been made on the changes in the distribution and quantity of fat in the body during and following exercise. Heron, Hales and Ingle (39) reported that all traces of body fat were absent after long periods of stimulation of rat muscles. Reed and his associates (53) studied the distribution of fat following exercise and found a decrease of fat in all the fatty depots with the exception of intermuscular fat. Haldi and his associates (38) compared the fat content of one group of rats which was allowed to exercise with that of another group kept in a state of rest. Although the exercising animals ate more than the non-exercising, the body weights of the two groups were the same at the end of the experimental period. On analysis more fat was found in the resting group. These authors concluded that the extra energy used by the

[1] A preliminary report of this work was made at the 51st Annual Meeting of the American Physiological Society, Toronto, Canada, April 29, 1939.

exercising group was supplied by fat oxidation. In similar experiments on dogs, Steinhaus, Hoyt and Rice (56) observed that the exercised dogs weighed less than their resting litter mates and the difference in weight was accounted for by the diminished amounts of subcutaneous and extra-organ fat. However, it is not justifiable to conclude from experiments of this nature that fat is being used for exercise. The fat found in the non-exercising group may have come from ingested carbohydrate which the exercising groups used as such, before it could be deposited as fat.

Not only may the distribution and amount of fat in the body be affected, but the ratio of phospholipids to cholesterol may change in exercise. Bloor and Snider (6) analyzed various muscles of the same animal and found that the muscles which performed the most work had a higher phospholipid content than the less active muscles, while the cholesterol content of these two groups showed only slight variations. The phospholipid content of muscles taken from wild rabbits was much greater than from laboratory animals. These results are suggestive that phospholipid is in some way related to muscular contraction.

Additional evidence has been obtained from the study of the respiratory quotient during exercise. In a review of the data from this source available in 1931, Carpenter (11) concluded that a mixture of metabolites was used for light muscular work. However, as muscular work progressed, a greater proportion of carbohydrate was oxidized. Finally, as the available carbohydrate decreased, fat oxidation became prominent. This theory would explain why the respiratory quotient for light muscular work remains the same as the basal, why it increases slightly during severe muscular work and then falls below the basal level as work is continued.

The only results at that time which did not fit into the general conclusions of Carpenter were those obtained by Best, Furusawa and Ridout (4). These observers reported quotients as high as 1.68 for the excess metabolism of work. Similar high quotients were obtained by Solandt and Ridout (55). The difficulty of calculating the excess quotient was discussed by Gemmill (29) and experiments were reported in which, with adequate recovery, the gross respiratory quotient after severe muscular work was comparable to the basal, and the

excess respiratory quotient was less than unity. Dill and his associates (23) found that the arterial carbon dioxide after severe work did not return to the normal level during a two-hour recovery period. When the respiratory quotients were corrected for this shift in carbon dioxide tension, the excess quotients for the entire period of work and recovery were between 0.9 and 1.0. Christensen and Hansen (13) have reported quotients of 0.95 with their subject working at such a rate that 4.15 liters of oxygen were used per minute. These experiments demonstrate that some men working under very severe conditions have respiratory quotients less than unity. Therefore, the results from severe work experiments may be incorporated in the general theory proposed by Carpenter.

Since the respiratory quotient is now regarded as the resultant of many metabolic events taking place in the body, which use oxygen and give off carbon dioxide, a respiratory quotient obtained during a short period of experimentation cannot be used to determine exactly the metabolic mixtures. Therefore, evidence for fat utilization based solely on respiratory quotient determinations is subject to this criticism.

Another approach to this problem has been the determination of the efficiency of muscular work in man on a high fat, or high carbohydrate diet. Although workers on a fat diet complain of fatigue and weakness during prolonged work, the actual mechanical efficiency of exercise in man is approximately the same on the two types of diet (Krogh and Lindhard (42), Reynolds (54), Marsh (45), Christensen and Hansen (12)). The recent work of Christensen and Hanson (12) is of special interest in this connection. They found that the net energy production in three subjects was about the same for work demanding 2.7 liters of oxygen per minute, but that the subjects could work two or three times as long on a carbohydrate diet as on a fat diet. These experiments do not give absolute evidence that fat is being used directly for muscular work since the fat may be converted to carbohydrate. Even if the conversion involves a loss of energy for muscular work, the decrement of energy may be used for other purposes and thus a measurement of the total exchange of energy would not reveal this conversion.

The increased acetone body production in blood and urine following

exercise has been used for evidence that exercise accelerates fat metabolism. An increase of these substances in the urine of man during and after exercise has been reported by Forssner (28), Preti (51), McClellan (46) and Christensen (12), and in the blood by Gemmill (30) and Courtice and Douglas (17). It would appear from these results that as exercise exhausts the carbohydrate supply, more fat is used, and the increase in the acetone bodies is a reflexion of this increased utilization. The difficulty of interpreting these data is that exercise decreases the carbohydrate stores, and therefore fat oxidation may be accelerated to meet basal requirements rather than to provide energy for work. Furthermore, blood and urine studies give only an index of the ratio of formation to utilization and tell nothing of the absolute magnitude of either process.

In isolated muscles, correlations have been made between oxygen consumption and carbohydrate loss during rest and work (Takane (58), Gemmill (31)). Since the carbohydrate loss does not account for the total energy exchange, the assumption may be made that the difference may be accounted for by fat oxidation. However, a similar assumption may be made for proteins or any number of other metabolites.

Although there are these many objections to circumstantial evidence, definite conclusions have been drawn from this type of evidence concerning the utilization of fat during muscular exercise. Dill (22) states that the evidence indicates that moderate work can be carried on for long periods on a fuel of fat. Christensen (12) calculates from his respiratory quotient data how much fat is used for exercise. Rapport and Ralli (52) in their study of exercise in phlorizinized animals claim that the fuel requirement in both rest and exercise is provided by the oxidation of fat. Many other examples of similar conclusions could be given.

In contrast to the large number of experiments supplying circumstantial evidence that fat might be used during muscular exercise, only a few reports of this utilization have come from direct determinations of fat in the muscle itself. Leathes (43) and Winfield (63) reported that stimulation of the frog's gastrocnemius had no effect on the fat content of the muscle, while Palazzolo (50), Niernierko (49) and Buchwald and Cori (10) found that the fat content decreased, especially

in frogs taken in the spring. The earlier work of Leathes, Palazzolo and Winfield may be criticised on the basis of inadequate methods. However, Niernierko and Buchwald and Cori did use modern techniques and, although both of their series were small and not all of their determinations showed a significant change, the average for the stimulated series was much less than for the controls. Thus their experiments must be accepted as showing a decrease in fat content of frogs' muscles following stimulation when the glycogen content of the muscles is low. For warm-blooded animals, no positive results have been reported. Culbertson (19), in cats, Buchwald and Cori (10), in rats, and Fenn and his associates (27), in cats, could not detect any evidence for utilization of fat. In fact, Fenn reported a slight rise in phospholipid content for the stimulated muscle when the results were reported on a dry-weight basis.

The evidence for fat utilization by cardiac muscle is in the same state as for cross-striated muscle. Cruickshank (18), from a critical evaluation of the literature available in 1936, concluded that fat was used during cardiac contraction. However, the evidence reported in his review was mainly indirect. Visscher (60) compared the fat content of a series of resting and working ventricular muscles. He found that the average fat content of the working muscles was less than that of the resting but owing to the edematous condition of the working muscle, it was impossible to make a definite statement that fat disappears during work.

In contrast to the lack of experimental proof for fat utilization in mammalian muscle is the complete accord of experimental data that carbohydrate is used for muscular work. The disagreement among workers in this field is not as to whether carbohydrate is used but as to whether it is used exclusively by muscle for obtaining the energy for muscular contraction. Analysis of isolated muscle after contraction, comparison of glucose analyses of arterial and venous blood, blood sugar determinations and many other types of experiment have demonstrated that carbohydrate is a fuel for muscular contraction.

However, it is well known that men and animals on a fat diet, and also diabetics can carry out considerable muscular exertion. The question then arises as to the nature of the fuel for muscular exertion when the carbohydrate supply is limited or when there is a disturbance

in its storage and utilization. With the hope of finding definite evidence for or against fat utilization by contracting muscle the present study was carried out by limiting the intake of food and increasing the output of glucose by phlorizin poisoning, with the idea that the carbohydrate stores in the body of the rat would be reduced to such a degree that fat oxidation during work would be increased to a measurable amount.

The amounts of fat and of carbohydrate which may be used during contraction were calculated from the data of Barcroft and Kato (2) obtained by stimulating the gastrocnemius of a dog at a rate of sixty times per minute. In their experiment No. 10, the resting oxygen consumption was 0.017 ml. per gram per minute and the average consumption during work was 0.062. The difference 0.045 multiplied by 60 gives an increment over the basal level of 2.7 ml. of oxygen used per gram of muscle per hour. This agrees with the values reported by Himwich and Rose (40a) in which tetanic contractions were used. They found an average resting value of 0.356 ml. per gram per hour and an average of 2.22 ml. during work. Converting 2.7 ml. oxygen per gram per hour into mgm. of fat which would be oxidized to use this amount of oxygen, the disappearance of fat would be 1.34 mgm. per gram of muscle. If conditions are such that fat is oxidized exclusively by the muscle, this value could be measured easily. However, if carbohydrate is used exclusively or in any proportion, the value would be diminished to such an extent that it could not be determined.

METHODS

Rats weighing about 250 grams each were selected. Phlorizin, c.p., in olive oil was injected subcutaneously in doses varying from 60 to 800 mgm. per kilo body weight. There were many fatalities from the larger doses of phlorizin. The rats were placed in individual cages and food was withdrawn for varying intervals before the experiments. Qualitative tests of reducing substances in the urine were made with Benedict's solution. In some of the control rats, food was also withdrawn but in the majority of experiments the rats were kept on their normal diet up to the time of the experiment. Following anesthesia with sodium pentobarbital (*Nembutal*), 38.4 mgm. per kilo injected intraperitoneally, the tibial nerves were dissected free and cut. In the stimulation experiments the nerve on the left side was stimulated by single maximal break-shocks delivered by a Harvard inductorium. The rate and separation of break from make shocks were controlled by a rotary key. The animal was held in position on a board by passing

cords around the legs and attaching the cords to the board. No attempt was made to record the tension developed but the contraction was roughly isometric as the attachment of the cords prohibited movement of the leg of the animal during contraction. The animal was placed under a lamp to prevent loss of body heat. The skin was sutured over the wound and wet gauze was suspended over the nerve to prevent drying of this tissue. In the control experiments in which the animal was kept on the board without stimulation similar dissection and nerve section were carried out.

For the fat determination the gastrocnemii were quickly dissected and corresponding parts of both muscles were cleaned of connective tissue and tendons. Following weighing on a torsion balance the muscle was ground in alcohol-ether (3:1) mixture with a small amount of sand. The alcohol-ether mixture, sand and mortar were previously cooled in ice. The contents of the mortar were transferred to a flask and the volume made up to 75 ml. with alcohol-ether mixture. The contents were refluxed for one hour over a boiling water bath, then cooled to room temperature and diluted to 100 ml. by additional alcohol-ether. After filtration, 50 ml. were analyzed for total fatty acids by the method described by Boyd (8) for the oxidative micro-estimation of these substances in blood. The term fat in this paper is used to denote the results obtained by the use of this method. A blank determination was made on 100 ml. of alcohol-ether mixture in order to obtain the base line for each experiment. The alcohol, ether and petroleum ether were redistilled before use. The peroxides were removed from the ether by treatment with potassium iodide and sulfuric acid (Bloor, 7). Ninety-four per cent recovery of 1.848 mgm. tristearin (Eastman) was obtained when the analysis was made by the complete method. The method of Cori and Cori (14) was used for the determination of total carbohydrate and that of Good *et al.* (33) for glycogen. In a few experiments the muscles were subdivided and corresponding parts used for both fat and carbohydrate determinations.

RESULTS

Nine experiments were made in which the content of fat in the corresponding gastrocnemii was determined without stimulation. In the majority of these experiments the muscles were taken directly from the animal after the rat was under anesthesia. Three experiments were carried out by allowing the animal to remain on the table under anesthesia for a time comparable to that of some of the stimulation experiments. In one of these, phlorizin was given to the animal two days previously and the animal was subjected to a fast for the same period of time. The average of all these experiments gave a close correlation between the fat content of the right and left gastrocnemii;

the percentage deviation being −7.5 per cent (Table 1). Three individual experiments, however, showed marked deviations with variations of +34.8, −43.5 and −36.0 per cent (Table 2).

The experiments in which one muscle was stimulated gave changes similar to those of the group in which both muscles were kept at rest (Table 3). Phlorizin and starvation did not accentuate the difference. The percentage change for all of the experiments was −4.0, while for those in which phlorizin was administered and the animals subjected to fasting it was only −5.3 per cent. Only one experiment (Dec. 20th)

TABLE 1

Average results of determinations of fat, of carbohydrate and of water contents of resting and stimulated muscles

DETERMINATIONS	DATA IN TABLE	NUMBER OF EXPERIMENTS	PER CENT WET WEIGHT			PER CENT CHANGE
			Stimulated muscle	Resting muscle, right	Resting muscle, left	
Fat						
All Determinations..	3	16	0.72	0.75		−4.0
Without Phlorizin.	3	6	0.73	0.73		0.0
With Phlorizin....	3	10	0.72	0.76		−5.3
All Determinations..	2	9		0.93	0.86	−7.5
Carbohydrate						
All Determinations..	4	11	0.40	0.51		−21.6
Without Phlorizin.	4	2	0.80	0.93		−14.0
With Phlorizin....	4	9	0.32	0.42		−23.9
All Determinations..	5	5		0.66	0.68	+3.0
Water Content						
All Determinations..	7	4	75.85	75.71		+0.2

gave a change exceeding the maximum difference observed in the resting group. With the average of the resting and the stimulated series falling in the same range, the only conclusion that might be reached from such results is that the fat content of the muscles was not decreased by stimulation.

In contrast, the results for the carbohydrate determinations showed a marked change in the stimulated muscle (Table 4). The general average for the stimulated group gave a percentage difference of −21.6, the phlorizin series −23.9 per cent and the normal group

TABLE 2

Fat Content of Resting Muscles

DATE, 1938	CONDITION OF ANIMAL			WET WEIGHTS OF MUSCLES		FAT CONTENT		DIFFERENCES
	Time on table	Phlorizin action	Fasting	Left	Right	Left	Right	
	hours	*days*	*days*	*grams*	*grams*	*per cent*	*per cent*	*per cent*
Nov. 14	0	0	0	0.388	0.313	1.28	1.36	−4.4
15	0	0	0	0.421	0.420	1.38	1.45	−4.8
16	0	0	0	0.395	0.371	1.32	1.42	−7.1
18	0	0	1	0.245	0.254	0.93	0.69	+34.8
21	0	0	0	0.420	0.386	0.64	0.60	+6.7
23	0	0	0	0.422	0.380	0.43	0.76	−43.5
Dec. 14	4.0	0	0	0.338	0.315	0.46	0.48	−4.2
27	2.0	0	0	0.440	0.350	0.48	0.75	−36.0
29	1.1	2	2	0.439	0.451	0.83	0.90	−7.8

TABLE 3

Fat Content of Stimulated Muscles

DATE, 1938–39	CONDITION OF ANIMAL				WET WEIGHT OF MUSCLES		FAT CONTENT		DIFFERENCES
	Time of stimulation	Rate per min.	Duration		Stim.	Resting	Stim.	Resting	
			Phlorizin action	Fasting					
	hours		*days*	*days*	*grams*	*grams*	*per cent*	*per cent*	*per cent*
Dec. 7	1.00	17	0	0	0.350	0.370	0.34	0.33	+3.0
16	1.00	22	0	0	0.402	0.445	0.68	0.60	+13.3
19	0.90	24	0	0	0.395	0.390	0.76	0.77	−1.3
20	2.00	24	6	1	0.321	0.332	0.25	0.77	−67.6
21	1.00	24	7	2	0.443	0.435	0.73	1.06	−31.1
22	1.00	24	8	3	0.417	0.411	0.66	0.80	−17.5
23	1.66	27	9	4	0.397	0.292	0.87	0.65	+33.8
28	1.66	26	0	1	0.455	0.462	0.96	1.00	−2.5
30	2.00	26	0	3	0.352	0.357	0.74	0.80	−7.5
Jan. 2	2.00	26	3	3	0.444	0.385	0.55	0.61	−9.8
3	2.25	64	4	4	0.346	0.350	0.55	0.49	+12.2
5	2.66	64	6	6	0.355	0.380	0.66	0.90	−26.7
Mar. 8	2.00*	80	0	0	0.397	0.393	0.89	0.86	+3.5
14	5.00	72	1	1	0.328	0.356	0.79	0.71	+11.3
15	6.66	78	2	2	0.404	0.416	1.12	0.82	+36.6
17	3.41	80	4	4	0.255	0.336	0.97	0.80	+21.2

* 2.0 hours stimulation and 2.0 hours recovery.

−14.0 per cent. The experiments are too few to allow one to decide if phlorizin accentuated the carbohydrate loss from the muscle. Under resting conditions, the deviation was +3.0 per cent with maximal differences of +7.7 to −3.8 (Table 5). Therefore the change in carbohydrate content of the muscle as a result of stimulation is a significant one. Similar results were obtained in the small group of rats kept on an olive oil diet (Table 6). It is interesting to note that

TABLE 4

Carbohydrate Content of Stimulated Muscles

DATE, 1939	CONDITION OF ANIMAL				WET WEIGHT OF MUSCLES		CARBOHYDRATE* CONTENT		DIFFERENCES
	Time of stimulation	Rate per min.	Duration: Phlorizin action	Duration: Fasting	Stim.	Resting	Stim.	Resting	
	hours		*days*	*days*	*grams*	*grams*	*per cent*	*per cent*	*per cent*
Jan. 20	1.66	64	0	0	0.391	0.315	0.82	0.95	−13.7
24	2.16	68	0	0	0.336	0.324	0.77	0.90	−14.5
26	2.00	68	2	2	0.422	0.382	0.08	0.36	−77.8
30	2.00	68	3	3	0.276	0.262	0.23	0.33	−30.3
31	2.00	68	4	4	0.366	0.407	0.57	0.70	−18.6
Feb. 1	2.00	68	5	5	0.369	0.422	0.66	0.73	−9.6
2	2.00	68	7	7	0.283	0.323	0.64	0.73	−12.3
Mar. 14	5.00	72	1	1	0.265	0.434	0.31	0.46	−32.6
15	6.66	78	2	2	0.364	0.352	0.20	0.24	−16.7
16	7.00	84	3	3	0.328	0.327	0.12	0.16	−25.0
17	3.41	80	4	4	0.373	0.428	0.05	0.09	−44.5

* Experiments of Jan. 20th to Feb. 2nd, total Carbohydrate determinations; experiments of March 14th to March 17th, Glycogen determinations.

after 13 days of this diet, the muscles and liver contained a considerable amount of carbohydrate.

Phlorizin and fasting lowered the carbohydrate content of the muscles but not the fat content. An average of the carbohydrate content in the resting muscles from animals receiving phlorizin compared to those not having this drug gave a decrease of −52.7 per cent. The majority of the animals receiving phlorizin showed a marked excretion of reducing substances in their urine, as determined by a qualitative test. The exceptions were rats used for the experiments of Dec. 22nd and 23rd. This result may have been due to the small dose of phlori-

zin given to these two animals and the long length of time after injection before they were used in these determinations. A similar comparison of the fat content of the muscles of the resting group receiving phlorizin and the normal series, showed only slight differences; the deviation between these two groups was only −9.4 per cent.

TABLE 5

Carbohydrate Content of Resting Muscles

DATE, 1939	CONDITION OF ANIMAL			WET WEIGHT OF MUSCLES		CARBOHYDRATE* CONTENT		DIFFERENCES
	Time on table	Duration		Left	Right	Left	Right	
		Phlorizin action	Fasting					
	hours	*days*	*days*	*grams*	*grams*	*per cent*	*per cent*	*per cent*
Jan. 19	0	0	0	0.338	0.342	0.98	0.91	+7.7
23	3.00	0	0	0.397	0.383	0.96	0.94	+2.1
25	1.83	1	1	0.432	0.476	0.50	0.52	−3.8
Feb. 6	2.00	3	3	0.404	0.395	0.50	0.48	+4.2
7	2.00	4	4	0.408	0.426	0.46	0.44	+4.5

* Total carbohydrate determinations.

TABLE 6

Determinations of Fat and Carbohydrate in Muscles from Rats on an Olive Oil Diet

DATE, 1939	CONDITION OF ANIMAL			WET WEIGHT OF MUSCLES		(F) FAT OR (G) GLYCOGEN CONTENT		DIFFERENCES	LIVER GLYCOGEN
	Time of stimulation	Rate per min.	Duration of diet	Stim.	Resting	Stim.	Resting		
	hours		*days*	*grams*	*grams*	*per cent*	*per cent*	*per cent*	*per cent*
Mar. 18	3	70	11	0.240	0.159	(F) 0.74	0.57	+29.8	0.09
23	2	76	13	0.246	0.228	(G) 0.16	0.39	−59.0	1.34
28	2	84	19	0.196	0.194	(G) 0.21	0.28	−25.0	0.15

These facts may also be used as indirect evidence that carbohydrate and not fat is being used by the muscles.

DISCUSSION OF RESULTS

The results given above are reported on a wet-weight basis. However, there is considerable evidence that the water content of a

stimulated muscle may differ from that of a resting muscle, and the magnitude of this change depends on the rate and duration of stimulation. It is obvious that a muscle stimulated at such a rate that lactate is formed, will draw water into its cells from the surrounding medium. The question arises as to the magnitude of the change in water content during moderate stimulation. Fenn and Cobb (26) stimulated indirectly the gastrocnemii of rats with stimuli causing tetanic contraction every second for periods of five to thirty seconds, and found that after bleeding the animal to death the water content of the stimulated muscles averaged 15.5 per cent more than that of the resting controls. Since bleeding an animal to death produces many profound alterations in the distribution of body fluids, it is difficult to believe that these changes were due to stimulation alone. In a few experiments these authors used muscles without resorting to bleeding and found that the stimulated muscle under these conditions actually had less water in it than was demonstrated by the former method. Another method would be to dissect the muscle from the animal, to control the hemorrhage, and to dry the muscle quickly between filter paper and to weigh it in a closed weighing bottle. It is realized that this method may not remove all the blood from the muscle, and since the stimulated muscle may have more blood present because of the dilated capillaries, this factor may be greater in the exercised muscle. However, the method affords a better comparison than when the animal is bled to death. Several experiments of this nature were made in order to determine the ratio of wet and dry weights under the conditions of the previous experiments in which fat or carbohydrate was determined. Dry weights were determined by placing the muscle in an oven at 105°C. for 18 to 24 hours and weighing after cooling in a desiccator. In a small series (Table 7), a maximal change of +1.16 per cent in water content was found after stimulation and a minimal of −0.64 per cent. Using the maximal value, the results of Dec. 30th were calculated on a dry basis. In place of 0.74 per cent for the stimulated, and 0.80 for the resting muscle on a wet basis, the values are 3.07 per cent and 3.24 per cent, respectively, on a dry-weight basis. The per cent difference between the two values is now −5.2 per cent in contrast with a difference of −7.5 per cent between the results calculated on a wet basis. This small difference

would not affect the general conclusions based on the results calculated on a wet basis and therefore it is considered valid to use the results as presented.

Another factor which must be considered is a change in the distribution of fats in the muscle. If only one fraction is oxidized, this use may be masked by the small change in the total analyses. The possibility of an increase in one or more fractions must also be considered and, in this case, the resultant effect may not indicate any change in total composition. However, from the calculations given above, if fats were used directly in a muscle, and to such an extent as the in-

TABLE 7

Water Content of Resting and Stimulated Muscles

DATE, 1939	CONDITION OF ANIMAL				WET WEIGHT OF MUSCLES		WATER CONTENT		DIFFERENCES
	Time of stimulation	Rate per min.	Duration		Stim.	Resting	Stim.	Resting	
			Phlorizin action	Fasting					
	hours		*days*	*days*	*grams*	*grams*	*per cent*	*per cent*	*per cent*
May 2*	0	0	0	0	0.4241	0.4371	75.78	75.86	−0.1
4	4.00	67	0	0	0.5153	0.4674	77.04	76.91	+0.2
6	3.50	70	0	0	0.3561	0.3733	76.01	75.14	+1.16
25	3.00	72	0	0	0.5020	0.5135	75.70	75.68	+0.03
30	3.00	84	4	4	0.3563	0.3238	74.63	75.11	−0.64

* Experiment of May 2nd: Resting control experiment. Animal was on table for two hours. Muscles were not stimulated.

direct evidence has led us to believe, the total content of fat should have shown a significant and measurable difference between the resting and stimulated series. Thus there was no need to fractionate the fats and the chemical and biological methods of analysis were sufficiently accurate.

Since there was no significant difference between the stimulated and resting muscles from animals even under the extreme conditions of fasting and phlorizin poisoning, many explanations might be given. These can be divided into the improbable and probable. Among the improbable are the possibilities that cutting the motor nerve inhibits fat oxidation and not carbohydrate utilization. Another possibility

is that blood fat may be used during exercise, as Needham (48) and Buchwald and Cori (10) have suggested. The fat in the muscle itself, under these conditions, would not change. This possibility, however, would demand an exact balance between utilization and supply; in fact, a more exact balance than for carbohydrate utilization, since carbohydrate in the muscle does decrease under similar conditions. Another unlikely possibility is that fat can only be used to supply the basal requirements of the body and does not serve as a fuel for muscular work. According to this idea the demand for carbohydrate would exhaust or diminish the supply during work and the fat stores would be called upon only to supply energy for basal requirements. Also, it may be possible that phlorizin inhibits fat oxidation or fat transport.

In contrast to these improbable explanations is the more probable idea that there was sufficient carbohydrate remaining in these animals to carry out the muscular contractions in these experiments. This carbohydrate may have come from previously ingested carbohydrate, from protein or from fat. The latter possibility suggests a way for fat utilization during exercise: it must be converted to a form which the muscles are able to use. Slowly data are accumulating for the proof of carbohydrate formation from fat. Gemmill and Holmes (32) demonstrated that liver slices from animals on a fat diet produced carbohydrate *in vitro* and that these slices had a low respiratory quotient. These two facts indicated new formation of carbohydrate from fat. The *in vitro* production of carbohydrate in liver slices has been confirmed by Cori and Shine (15), Bach and Holmes (1) and Stadie (57) and the low respiratory quotients by Elliott (25). Cori and Shine, however, gave a different explanation of these results. They claimed that since free phosphate was formed in the reaction the new carbohydrate might be coming from a glycerophosphate, and they showed that the addition of glycerophosphate increased the carbohydrate content of incubated liver slices. This may be a possibility but it would not explain the low respiratory quotients found in these experiments. Haarman (36, 37) claimed that β-hydroxybutyrate may be converted to lactate in tissue slices. Better evidence was obtained by Weil (61) in kidney slices in which the carbohydrate was chemically isolated and determined. Blixenkrone-

Møller (5) has recently reopened this question, using liver perfusion as a technique, and has claimed that a large percentage of added butyrate is converted to sugar by the liver. Brand and Krogh (9) measured the respiratory quotient and the carbohydrate formation in starved rats following work. Since the rise in carbohydrate content of the muscles was associated with a low respiratory quotient during the recovery period, these authors concluded that the source of the carbohydrate might be fat. Although the amount of carbohydrate coming from fat may be very small, the possibility of this conversion must be considered in any physiological process and it cannot be dismissed by any dogmatic statement that fat cannot be converted into carbohydrate.

Another possibility for fat utilization is that fat may be converted into ketone bodies which the muscle may use in a working, as well as in a resting condition. Thunberg (59) with his methylene blue technique demonstrated that β-hydroxybutyrate dehydrogenase was present in muscle. Green, Dewan and Leloir (35) made a thorough study of this enzyme from muscle and found that the system dehydrogenase + coenzyme I + dl-β-hydroxybutyrate took up molecular oxygen and the hydroxybutyrate was oxidized to acetoacetate. Edson and Leloir (24) have described a similar oxidation in muscle slices. Mirsky (47) has demonstrated disappearance of acetone bodies in the eviscerated animal and Barnes and his associates (3) have shown the same in the heart-lung preparation. Evidence that acetone bodies are used in muscular contraction has been given by Blixenkrone-Møller (5) who demonstrated that a large amount disappeared from the blood after passage through stimulated muscles.

It will be interesting to speculate how acetone bodies can be used for muscular contraction. The current scheme of carbohydrate breakdown gives the first oxido-reduction at the triosephosphate stage. The triosephosphate is converted to phosphoglycerate with reduction of the coenzyme. This process is associated with rephosphorylation which in turn supplies more energy for the contractile process. Since β-hydroxybutyrate + dehydrogenase also can reduce the coenzyme and since this system can take up oxygen, it may be at this point that the energy is supplied to the muscle. The conversion of pyruvate to acetoacetate (Krebs and Johnson (41)) is also of interest in this con-

nection. If this reaction can be shown to be reversible, the pyruvate formed from the acetoacetate may be converted into lactate. Dewan and Green (21) have shown that the oxidation of β-hydroxybutyrate to acetoacetate is coupled with the reduction of pyruvate to lactate. Thus, β-hydroxybutyrate may take the place of triosephosphate in the current scheme of glycolysis and of muscular contraction. The connection of these events with phosphorylation would be needed to make the analogy complete.

The glycerol phosphate fraction of phosphatides is readily metabolized and this gives another possibility for the utilization of fat. Although this substance is only a small fraction of the total fat, considerable energy may be obtained from its oxidation. It may be converted into carbohydrate in the liver (Cori (15)) or into triosephosphate in the muscle (Green (34)). It is well known that glycerol may also be converted into carbohydrate. Much more work is needed in enzymatic solutions and in intact tissues before a definite answer can be obtained regarding the indirect utilization of fat in muscular exercise.

Evidence is also accumulating that a phlorizinized animal uses carbohydrate. Wierzuchowski (62) obtained recoveries varying from 71 to 95 per cent of the ingested glucose in a phlorizinized dog and claimed that the difference was due to oxidation of glucose. Deuel, Wilson and Milhorat (20) have also shown that as much as 20 per cent of the ingested glucose under these conditions disappears due to oxidation. It may act as an inhibitor of glycolysis (Cori (16), Lundsgaard (44)), but only in large concentrations. Both Cori and Lundsgaard remark that the concentration of phlorizin required for inhibition of glycolysis is so great that this inhibition is never produced in animal tissues with the possible exception of the kidney. Direct evidence that glycolysis is not inhibited in the intact animal has been supplied by Himwich and his associates (40b) who have shown that lactate production takes place during exercise in the phlorizinized animal. The present experiments demonstrate that the carbohydrate content of stimulated muscles in phlorizinized rats diminishes. Whether the decrease is due to a conversion of carbohydrate to lactate or to an oxidation of carbohydrate has not been determined in these experiments.

This type of experimentation does not give the absolute amount of carbohydrate used during stimulation but shows that it is decreased in active muscles. In all experiments the disappearance of carbohydrate was much less than could be accounted for by the calculation of total oxidation under similar conditions and therefore additional carbohydrate, which was carried to the muscles by the blood, may have been used.

Although no quantitative record of tension developed during stimulation was made, it was observed that the phlorizinized fasted animals gave weaker responses and, in some experiments, the stimulation had to be discontinued because the muscle refused to respond. Diminished response in muscles with decreased carbohydrate content has been described and the weakness and early fatigue of men on fat diets has been observed frequently. Therefore, if the indirect mechanisms described above for the utilization of fat for muscular exercise are used by the body, they are not as efficient in preventing fatigue in prolonged exercise as direct carbohydrate oxidation.

SUMMARY

Experiments were designed to decrease the carbohydrate supply in the body of a rat in order that fat oxidation in the muscle during exercise might be accelerated to a measurable degree. This result was not achieved. The averages of the fat determinations show no significant difference between the resting and stimulated muscles even after phlorizin poisoning and fasting. In contrast, the carbohydrate content of the stimulated muscles decreased both in the normal and phlorizinized animals. When fat is used to supply energy for work in mammalian muscle, it is used indirectly. A discussion is given of the possible indirect pathways of utilization of fat for energy production during exercise.

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THE USES AND LIMITATIONS OF SOFT TISSUE ROENTGENOGRAPHY IN PLACENTA PRAEVIA AND IN CERTAIN OTHER OBSTETRICAL CONDITIONS

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Placenta praevia is one of the most serious complications of pregnancy and labor. If the presence or absence of this condition can be accurately established in a patient presenting herself with a history of vaginal bleeding, the future course of the pregnancy can be more accurately and safely outlined. We have found that the radiologist can be of invaluable aid in arriving at a diagnosis of placenta praevia by employing a method which avoids the inherent dangers of a vaginal examination. We believe that this method may be employed successfully in a high percentage of cases, and herewith present our experience in cases of placenta praevia, suspected placenta praevia, and other obstetrical conditions. The method is not new, yet it has been employed, to our knowledge, by only two other groups of investigators.

The method, as used by us at the present time, consists of two separate examinations, and these will be discussed in turn. The first step in the procedure is the production of a single lateral 14 in. *x* 17 in. film of the uterus, taken by means of a technique designed to furnish the maximum of soft-tissue detail. This method of examination was first reported by Snow and Powell (9) in 1934. It has also been employed for a number of years by Smith (8).

The exact technique must be determined by trial and error in each roentgenological laboratory, for it must vary with the equipment at one's disposal. Roughly, the technique employed in our laboratory has been as follows: The K.V.P. is determined from the actual measurement of tissue thickness and is varied between 65 and 82 (peak kilovolts), depending upon the obesity of the patient. The milliampere-second factor is constant and is 200, the factor being made up of 50 M.A. for four seconds. Movement of the foetus during this

long exposure does not interfere either with the production of a good film or with the interpretation of the same. A tube-film distance of 42 inches is employed with the Bucky diaphragm and Par Speed screens. All films are developed, fixed and washed under accurate time and temperature control. No filter is employed.

Since mild anoxemia in the mother causes motion of the foetus, the mother is not coached in holding her breath before the actual exposure of the film is made.

The lateral film obtained by this method, we believe to be the most important one in the study of the uterus and the product of gestation. Its correct interpretation requires not inconsiderable study and practice. If the film is properly taken, the posterior uterine wall may be clearly visualized with the aid of any viewing box, but the anterior abdominal and uterine walls, which represent a much thinner area of tissue penetrated by the x-rays, will be "burned out," i.e., over-exposed. However, by viewing this latter area with a concentrated light, as with a 200-watt frosted bulb in an ordinary goose-neck lamp, with a shade, it can be as clearly made out as the posterior uterine wall and the latter's adjoining soft and osseous tissues.

The placenta is localized in the region where the structures between the outer uterine wall and the foetal soft parts are definitely increased in thickness. The cutaneous region of the foetus is usually represented by a dark line, due to radiolucency and variously ascribed to vernix caseosa, subcutaneous fatty tissue, and photographic effect. Irrespective of the origin of the dark line, it is very helpful when present, yet not essential to correct localization of the placenta. Snow (10) has pointed out that the ventral surface of the foetus is directed toward the placenta and that its extremities frequently produce indentations into the foetal surface of the placenta. We have not observed this regularly, and do not believe that it is of absolute diagnostic value. Another point which has not, to our knowledge, been previously noted, is the relative distance of the nearest foetal part from the sacral promontory or lumbar spine. Not infrequently this will furnish information on the possibility of implantation of the placenta in the intervening region, whether it be the region of the posterior wall of the lower uterine segment or of the fundus.

It is impossible to demonstrate all of the foregoing features in a single illustration unless certain photographic devices are used. Since it is desirable that illustrations show all these features, we shall describe the manner in which the accompanying photographs were made, so that they may be properly interpreted. Figure 1 is a direct reproduction of a properly made film. It will be noted that the anterior wall shows up poorly. Figure 2 shows the same film reproduced to show both walls by using a small amount of light passing through the posterior uterine wall and a larger amount through the dark anterior wall.

Some difficulty in placental localization might be expected when there is no apparent thickening of the anterior or posterior uterine wall. Since the lateral view shows the uterus in profile, it is the midplane which is specifically reproduced. If the placenta, then, does not cover an appreciable amount of the anterior or posterior surface, i.e., if it is implanted over one or the other lateral wall of the uterine cavity, there will be little or no apparent thickening of the myometrium. However, this is a very rare occurrence. By a new method of studying placentation, Torpin (13) has shown that in the great majority of cases, the placenta is implanted over the anterior or posterior uterine wall. Again, most placentae are large enough (average 25 to 30 per cent of internal uterine surface) so that even if primarily implanted over a lateral wall, they would extend far enough over the anterior or posterior wall to be visualized in the lateral roentgenogram. This fact also explains why any placenta located below the equator of the uterus may reach as far down as the margin of the internal cervical os, or may even partially or completely cover it. The possibility of such abnormalities of implantation as succenturiata, bipartita, and spuria, must always be borne in mind when ruling out the presence of placenta praevia from this single lateral roentgenogram.

The most common factor which hinders or prevents localization of the placenta by this examination is hydramnios. Excessive obesity, too, has on several occasions made localization impossible. If the foetus is so premature that the subcutaneous fat is insufficient to produce the dark line outlining its soft tissues, and the relative volumes of fluid and foetal mass are such that a condition similar to hydramnios is produced, the accurate localization of the placenta is interfered

FIG. 1. UNDODGED NEGATIVE TO SHOW APPEARANCE OF FILM WHEN VIEWED ON THE ORDINARY LIGHT BOX

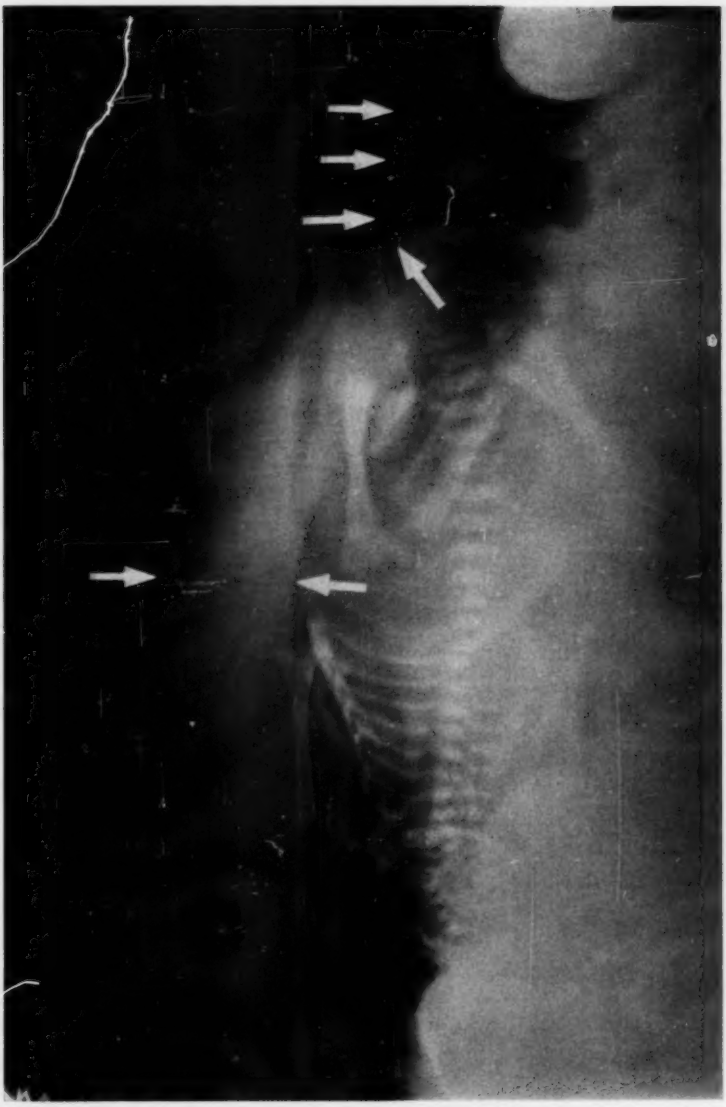

FIG. 2. SAME FILM REPRODUCED WITH DODGING TO PERMIT DEMONSTRATION OF BOTH UTERINE WALLS ON ONE NEGATIVE

Usually the posterior wall would be seen on the light box and the anterior wall seen by viewing with 200 watt bulb. The placenta is seen on the anterior wall of the uterus.

From above down the arrows point to (1) anterior abdominal wall; (2) abdominal musculature; (3 and 4) uterine wall, normal thickness; (5 and 6) uterus plus placenta giving localized thickening.

FIG. 3. PLACENTA WELL UP IN FUNDUS POSTERIORLY

Upper arrows indicate uterine silhouette. Lower arrows indicate nearest fetal soft parts, placenta causes localized thickening at this point.

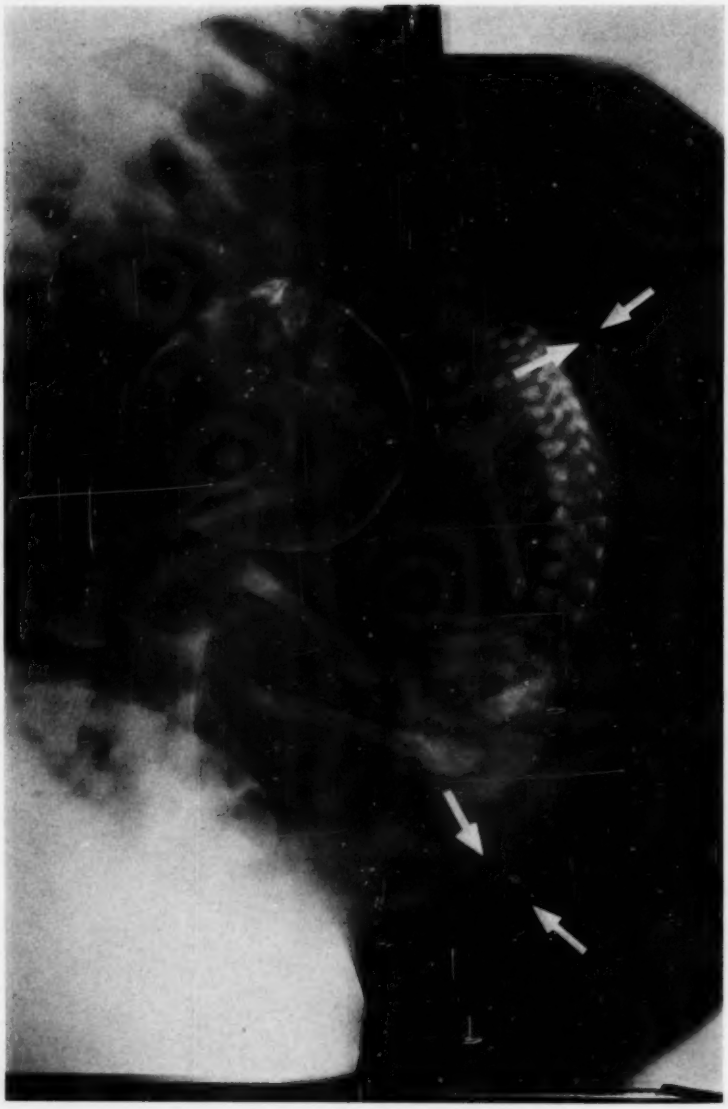

FIG. 4. PLACENTA LOW ON ANTERIOR WALL, PROBABLY REACHING CERVICAL OS. CONFIRMED AT SECTION

Upper arrows indicate normal thickness uterine wall. Lower arrows localized thickening due to placenta. Abdominal wall at base of point of lowermost arrow.

with. Again, multiple pregnancy, even when not associated with hydramnios, renders the accurate diagnosis difficult. The presentation of the foetus need not be an interfering factor, although the localization is more easily made with longitudinal than with transverse presentations. Likewise, breech position may occasionally cause some difficulty in accurate localization.

The second method of examination consists of an anteroposterior and a lateral roentgenogram, each 14″ x 17″, of the pelvis, with air in the bladder, as advocated by Snow (9, 10, 11 and 12). The anteroposterior film is obtained by employing the same factors as described above for the lateral soft tissue roentgenogram. The second lateral film, on the other hand, is obtained with technique comparable to that used in taking a lateral film of the lumbar spine, since it is intended to demonstrate only the soft tissue layer separating the air-filled bladder from the presenting foetal part. Cystography has been employed by many for the diagnosis of bladder lesions and certain other pelvic conditions which produce changes in the shape of the bladder. Its aim, as utilized by Snow and by us, is to determine the thickness of soft tissue separating the foetal head from the fundus of the air-filled bladder. This tissue is normally not in excess of two centimeters in thickness, when films are made at 42 inches' distance, and in late pregnancy it is usually somewhat less thick. In the presence of central placenta praevia, the distance between the presenting foetal part and posterosuperior wall of the bladder, as determined from either anteroposterior or lateral films, or both, may be increased to as much as five or six centimeters. With marginal placenta praevia, on the other hand, the increase in tissue thickness may be localized either laterally or anteriorly. Obviously, a placenta attached to the posterior wall of the lower uterine segment, unless it extends over the cervical os, cannot produce any thickening of this tissue layer.

We believe that air in the bladder presents distinct advantages over the use of opaque media, as advocated by Ude and Urner (14). The amount of media in the bladder may be increased from 20 or 30 cc. of sodium iodide to 100 or 150 cc. of air without producing a bladder shadow that will overlap and partially obscure the foetal shadow. The air will produce a double shadow, one for the anterior, thinner and

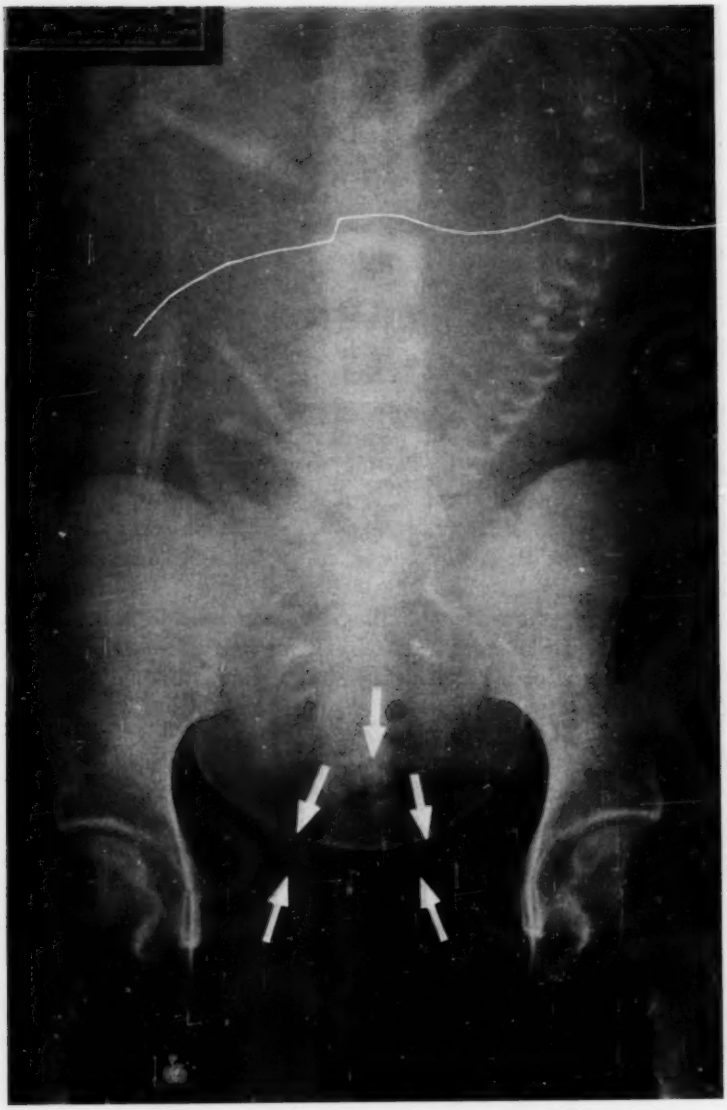

FIG. 5. NORMAL RELATION OF FETAL HEAD TO AIR-FILLED (150 CC.) BLADDER

Upper arrow indicates the shadow cast by the anterior reflection of the bladder. The middle two arrows indicate the fetal skull and the lower arrows the dome of the bladder.

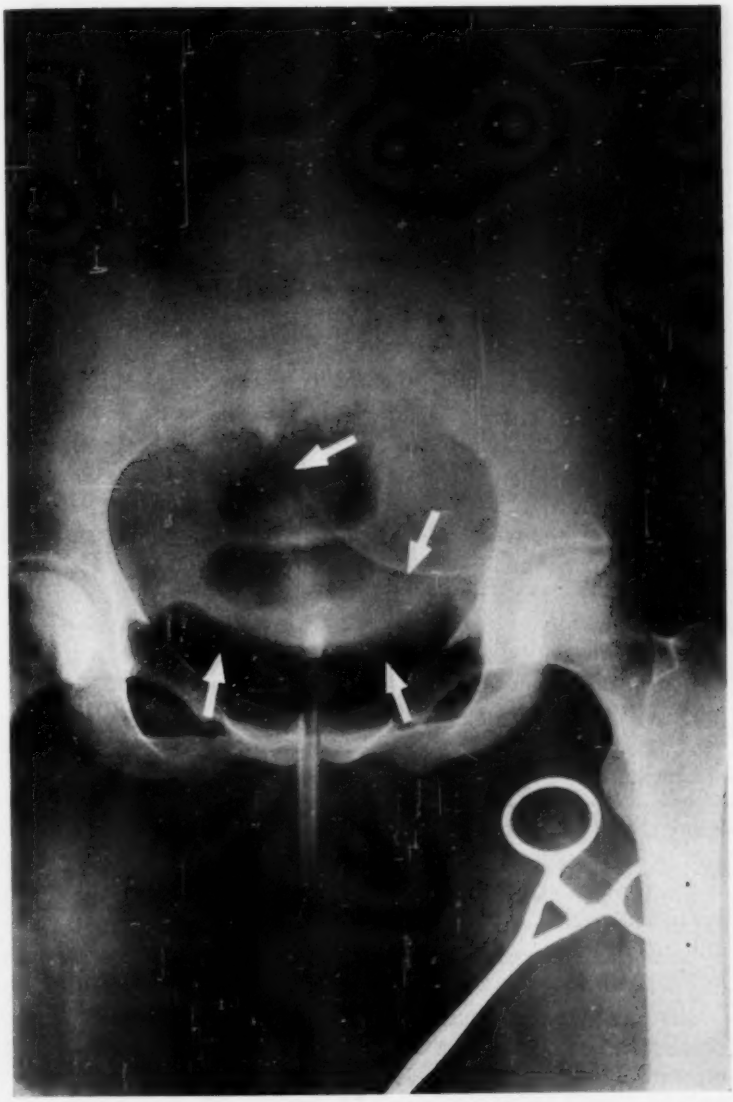

FIG. 6. TYPICAL APPEARANCE OF PLACENTA PRAEVIA WITH AIR-FILLED BLADDER

Upper arrows on fetal skull. Lower arrows indicate dome of bladder. Placenta produces marked thickening of intervening soft parts.

higher portion of the bladder, and a second and denser one for the lower region that is encroached upon by the uterus. Again, air has been found to cause the bladder to mould itself more closely to the uterus when a foetal part other than the head presents. Lastly, a lateral view can be obtained. This is of distinct value in certain cases. However, it is entirely possible that our preference for air rather than opaque media is a matter of personal choice and experience, since those who use opaque media seem to be just as successful in making or ruling out the diagnosis of placenta praevia, at least when central placenta praevia is present.

There are certain factors which may lead to errors in interpreting the films that have been obtained with air in the bladder. Of these, an excess of gas or feces in the rectum can be controlled. Only occasional extra-uterine pelvic or abdominal masses are troublesome. Prematurity is a definite factor, as has been pointed out by Ude and his co-workers. Premature separation of the normally implanted placenta, with accumulation of blood clot in the lower uterine segment might produce the roentgen characteristics of placenta praevia. Moreover, we have observed one case in which the bladder-foetal head space was increased without known cause, but the possibility of placenta praevia had been ruled out through localization of the placenta in the fundus, by means of the soft tissue lateral roentgenogram. The method in which air is injected into the bladder can be employed to advantage only in the cases in which the foetus is in a vertex position.

While we consider the lateral soft tissue film the most valuable one, it must be emphasized that the roentgenograms obtained with the air-injected bladder are occasionally of definite aid. For instance, in cases of hydramnios, where it is impossible to visualize the placenta by soft tissue roentgenography, the presenting part of the foetus may lie so close to the bladder that placenta praevia can be ruled out.

Our procedure at present is to obtain routinely the lateral soft tissue film. If this arouses any suspicion of low implantation of the placenta, or if this examination is unsatisfactory, the examination with air in the bladder is made. On the other hand, if there is a history of vaginal bleeding or if the clinical examination strongly suggests placenta praevia, then all three films are obtained at once.

Patients with frank bleeding, who are in critical condition, are never subjected to the danger of moving and handling necessary for the roentgenographic examination.

During the course of this study, we have attempted several modifications which theoretically should improve the quality of the soft tissue films, but which have proved disappointing on our hands. We mention these, since they may prove to be valuable adjuncts in the hands of others attempting to refine this method or develop a technique in their laboratories. Early in the study, we found that the use of an aluminum filter resulted in loss of detail, and have since employed unfiltered radiation. We attempted to equalize the density of the film at the regions of anterior and posterior uterine walls by inserting a lead paste wedge between the x-ray tube and the patient to shield the anterior uterine wall, but found that the inclusion of any type of filter resulted in loss of soft tissue detail. In a few instances non-screen films were employed, but the resulting reproductions were inferior.

While the chief aid of this roentgen method to the practice of obstetrics lies in the diagnosis or elimination of placenta praevia, there are other uses which might occasionally arise. Its possible use in cases of premature separation of the normally implanted placenta has been mentioned. The diagnosis of this condition could conceivably be made on the basis of the soft tissue film (11, 12). Localization of the placenta previous to the insertion of bougies for the induction of labor, allows the obstetrician to select the site at which the insertion of the bougies will be least likely to cause separation of the placenta. If the exact location of the anteriorly implanted placenta is known previous to Caesarean section, the operator might care to avoid the excessive bleeding attendant upon separation of the placenta before extraction of the foetus, by choosing a classical or a low cervical incision, depending upon whether the placenta is located low or high on the anterior wall. There are some obstetricians who are of the opinion that the placenta implanted over the region of a previous Caesarean wound, predisposes to rupture of the uterus. In these an elective Caesarean section would be desirable, as has been pointed out by Snow and Rosensohn (11). Again, if the placenta is not implanted over the region of the old wound, it is possible, in some

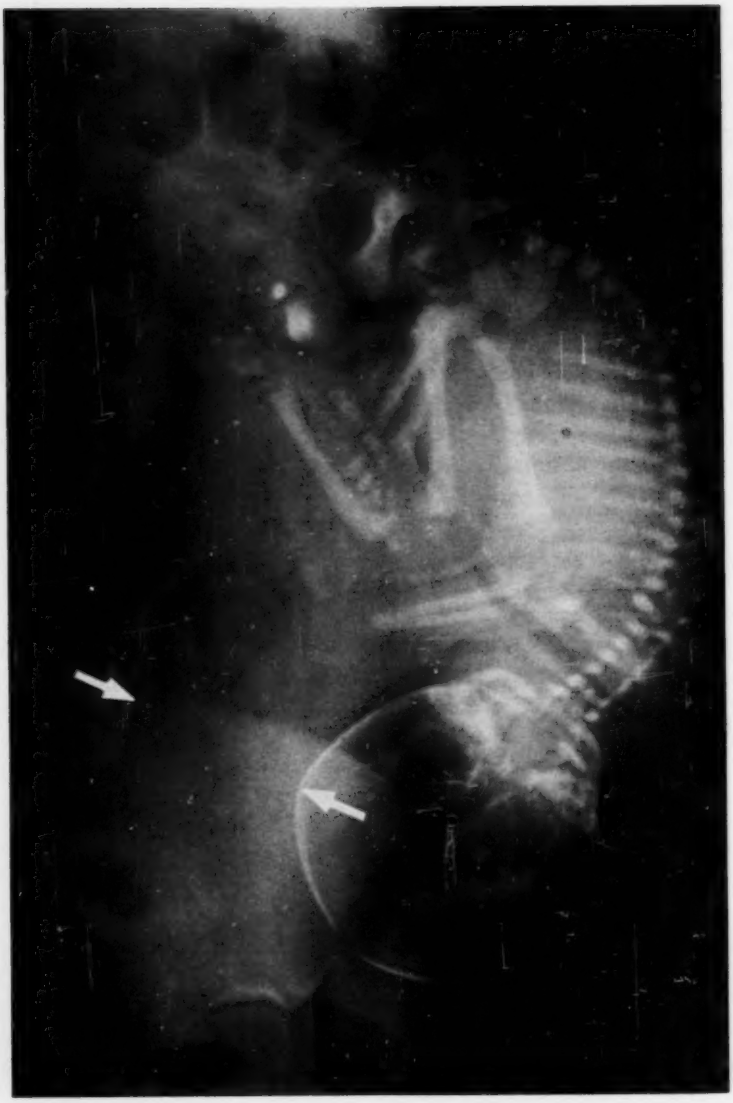

FIG. 7. PLACENTA PRAEVIA WITH PLACENTA ON POSTERIOR WALL
Note marked separation of fetus and maternal spine

FIG. 8. EXTRA UTERINE PREGNANCY

Note pathognomonic thin-walled sac.
Upper arrows indicate sac. Lower arrows placenta.

cases, at least, to obtain information on the relative thickness of the uterine wall at the site of the previous incision. This has been accomplished once in our experience and was responsible for the patient's coming to section with confirmation of the diagnosis of a thinned-out scar and subsequent repair. Advanced extra-uterine pregnancies may be diagnosed by this method, and Snow (12) has done this successfully in six instances. Finally, we have found it advantageous to use the soft tissue lateral examination when roentgenograms are requested for suspected foetal anomalies, abnormal presentations, foetal death in utero, and even multiple pregnancy and hydramnios. The soft tissue film furnishes good detail of osseous structures so that in addition to supplying information concerning location of the placenta, it may also be of use to obtain other information desired by the obstetrician.

It has been possible to localize the placenta in over 85 per cent of more than 200 cases examined. The percentage of success would be higher if we eliminated films which were taken under adverse circumstances, i.e., in cases in which the foetus was too young and in cases in which the films were made for the purpose of demonstrating myomata which, of course, cannot be demonstrated unless the myomata happen to contain calcium.

In 53 cases in which the presenting symptom was vaginal bleeding, the diagnosis of placenta praevia was made or ruled out in 44. In 10 of the 44 the diagnosis was positive and in 34 negative. In 9 of the 53 cases the diagnosis could not be made either positively or negatively owing to interfering factors of the type cited above. In the ten cases in which a positive diagnosis of placenta praevia was made, this was confirmed at the time of delivery. In the 34 cases in which the placenta was localized elsewhere than at the cervical os, this localization was also confirmed.

In no instance so far has the placenta been found, either at delivery or during Caesarean section to lie at any other site than that at which we have localized it.

SUMMARY

1. The site of implantation of the placenta can be accurately localized by soft-tissue roentgenography in a large percentage of cases.

2. The accuracy of the localization may be improved in some

cases by making additional roentgenograms after the bladder has been distended with air.

3. The use of these methods of localization is a very valuable aid to the obstetrician in cases of suspected placenta praevia and other obstetrical conditions.

4. The procedures and diagnostic considerations, as well as factors which might lead to errors in interpretation, are discussed and illustrative cases are presented.

5. The results obtained by this method are presented.

6. It is believed that with increased experience and greater attention to detail even better results may be anticipated.

Since the preparation of the manuscript, it has come to our attention that Weintraub, S. and Snow, W. (Am. J. Roentgen. & Rad. Therap. **42:** 718, 1939) have quite conclusively proven that the dark line at the borders of the fetus is due to subcutaneous fat.

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STUDIES ON THE SITE OF SENSITIVITY IN THE ARTHUS PHENOMENON[1]

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During infection the body commonly becomes sensitized to the proteins liberated from the infecting bacteria. These proteins may be quite harmless for the normal body, but when the state of hypersensitivity is established they are able to cause damage and death of tissue, accompanied by acute inflammation. Likewise, if a foreign protein which is innocuous to the normal body be injected repeatedly into the tissues, a state of hypersensitivity results which renders the bland protein capable of producing damage and death of tissue and acute inflammation. The hypersensitivity established through infection is ordinarily termed "bacterial hypersensitivity" or "hypersensitivity of infection"; that caused by the repeated injection of soluble foreign proteins is known as the "Arthus type of hypersensitivity."

The inflammatory-necrotic lesions that result from contact of the sensitized tissues with the specific proteins in these two types of hypersensitivity may resemble each other so closely that the two states have frequently been confused, and erroneous conclusions of both theoretical and practical importance have resulted from the confusion. For example, when tuberculoprotein is injected intracutaneously in testing for the presence of tuberculous infection in the human being, if the test injections are repeated after an initial negative test, reactions may occur which have been interpreted as positive tuberculin tests indicative of tuberculous infection. More careful study, however, shows that these reactions are not typical tuberculin reactions, but have characteristics of Arthus reactions. They do not signify the presence of tuberculous infection, but are due rather to the establishment of Arthus type sensitivity as a result of the injections of tuberculoprotein. A description of such reactions may be found in the report of Aronson and Nicholas (8).

[1] Presented in abstract at the Third International Congress for Microbiology, New York City, Sept. 4, 1939.

Again, the repeated injection of tuberculoprotein into animals produces a state of hypersensitivity in which the intracutaneous injection of the protein evokes a local hypersensitive reaction. This sensitization has been regarded as being "of as high degree and of the same type as that produced by infection with live tubercle bacilli" (15). Since animals sensitized in this manner develop no resistance to tuberculous infection (16, 17), it has been implied that the bacterial type of hypersensitivity has been established without the concomitant development of acquired resistance. This conclusion, if valid, would be of fundamental importance from the standpoint of the problem of the rôle of bacterial hypersensitivity in resistance to infection. However, a closer examination of the matter shows that the animals sensitized by the injection of tuberculoprotein differed in important ways from animals sensitized as a result of infection. They did not, for example, react hypersensitively either to tubercle bacilli or to Old Tuberculin (14, 18); and there is evidence that the characteristics of the reactions in animals so sensitized resemble those of the Arthus type rather than of the bacterial type of hypersensitivity (19, 8).

These examples will serve to indicate that, in their developed state, the Arthus reaction and the local reaction of the hypersensitivity of infection may resemble each other closely enough to lead to confusion, and this resemblance has led, in some quarters, to the belief that there may be no really fundamental difference between the hypersensitivity that occurs during infection and that which results from the repeated injection of any foreign protein. Most students of the subject, however, prefer for the present to separate the hypersensitivity of infection from the Arthus type of sensitivity, for there are several rather striking differences between these two states. Chief among these differences are, first, the fact that the local and constitutional reactions produced by contact with the specific protein develop more rapidly in the Arthus type of sensitivity than in the hypersensitivity of infection; and second, that the Arthus type of sensitivity is readily transferable passively to a normal animal by injecting into it the serum of a hypersensitive one, whereas the hypersensitivity of infection is not transferable passively. Since exceptions to these and to other differences between the two states have been observed, it has been a matter of question whether the differences really imply fundamental differences

in the mechanism of the two types of hypersensitivity. Because of the important implications in relation to the problem of the significance of hypersensitivity in infection, it is desirable to gain as much information as possible relating to the fundamental identity or nonidentity of these two manifestations of the hypersensitive state.

Several years ago Rich and Lewis (1) showed that the damage and death of tissue which the bacterial protein produces in the body sensitized by infection is the result of a sensitization of the individual body cells. When the cells of the blood or tissues were isolated from the sensitized body, washed, and brought into contact with the bacterial protein in tissue cultures, they were damaged and killed by amounts of the protein which did not harm the cells from the normal body. Aronson (2), and Moen and Swift (3) have confirmed these observations, and the former investigator, in similar studies (4) showed that, in contrast, the cells of the Arthus sensitized body are not injured *in vitro* by contact with the specific protein. That observation we can substantiate. The cells of Arthus sensitized animals are not damaged *in vitro* by contact either with the specific protein or with a mixture of the protein with its antibody (5). Furthermore, in collaboration with Mrs. Warren H. Lewis and Dr. Leslie N. Gay, we have recently shown that in patients with asthma and hay fever there is no generalized cellular hypersensitivity to the specific protein, such as exists in the hypersensitivity of infection. In patients of this type, who were so hypersensitive that the injection of a very minute amount of the specific protein produced a severe and extensive local lesion, the cells, when removed from the body, were unharmed by contact *in vitro* with even high concentrations of the protein (5).

This, then, appears to be a fundamental difference between the hypersensitivity of infection and the Arthus and "pollen" types of hypersensitivity. In the former, the local tissue damage produced by the protein is due to a sensitization of the individual tissue cells; in the Arthus and "pollen" types of hypersensitivity there appears to be no generalized cellular hypersensitivity. How, then, is necrosis of tissue produced in the Arthus reaction, if the tissue cells at large are not sensitized? The purpose of the present experiments was to attempt to gain information regarding the site of sensitivity and the mechanism of the tissue damage in the Arthus reaction.

There are reasons for believing that the blood vessels may be specifically sensitized in the Arthus type of sensitivity, and that the tissue damage may be due to local ischaemia resulting from the rupture and thrombosis of sensitized vessels. It has long been noted that vascular damage is a prominent feature of the histopathology of the Arthus reaction (9, 10). The haemorrhagic nature of the reaction was noted by Arthus in his original reports (13), and no one can study the development of the reaction histologically without being impressed by the early vascular damage. Endothelial cells of many of the capillaries have karyorrhectic nuclei; in many cases the necrotic wall has ruptured, spilling blood into the tissues; other capillaries are plugged with thrombi made of masses of leucocytes or of coalesced red cells. Abell and Schenck (20) have observed the occurrence of these effects directly in transparent chambers in the sensitized rabbit's ear. That local ischaemia results from these vascular alterations is obvious; but is the death of tissue caused solely by ischaemia, or are the tissue cells of the sensitized body directly damaged and killed by contact with the specific protein? Opie (10), from experiments that will be described in the Discussion of the present paper, concluded that in the Arthus sensitized body the tissue cells are directly damaged by contact with the precipitate that forms when the specific protein meets the precipitin in the tissue fluids. The *in vitro* studies mentioned above speak against a direct necrotizing action of the foreign protein or its specific precipitate upon the extravascular tissue cells, but it was desirable to have the matter tested in the intact body. The plan of the present study, therefore, was to determine in the first place whether, in the Arthus sensitized body the cells of a tissue that normally contains no blood vessels would be killed by contact with the specific protein; and, if they were not killed, to learn whether an Arthus reaction could be produced in that tissue if blood vessels were first caused to grow into it. For this purpose we chose the cornea as a readily observable avascular tissue. Since the Arthus type of sensitivity is well established as being dependent upon antibody (that it is passively transferable has been mentioned above) it is important to state that when antibodies are present in the blood they penetrate into the normal cornea. This has been demonstrated in the case of precipitins, hemolysins, agglutinins and antitoxin (11, 12).

THE TUBERCULIN REACTION IN THE CORNEA

For purposes of comparison, it was first desirable to determine whether the effects of bacterial hypersensitivity are demonstrable in an avascular tissue. To test this point we injected tuberculoprotein into the cornea of tuberculous, hypersensitive guinea pigs and of normal controls. In order to avoid the irritant effect of the glycerin that is present in Old Tuberculin, tuberculoprotein was obtained by precipitating a water extract of pulverized, heat-killed tubercle bacilli with ammonium sulphate, washing the precipitate, and redissolving it in water. The activity of the solution was equal to that of potent Old Tuberculin. In each case a minute drop of the undiluted solution was injected into the substance of the cornea near the center. The animals were killed with ether at the end of 24 and 48 hours, and the eyes were removed and fixed in Zenker-formol, the sites of injection being marked by pins placed in appropriate positions in the sclera. After hardening, the cornea was cut into strips, and numerous microscopic sections were made of each strip.

The difference between the effect of the tuberculoprotein upon the cornea of the normal and of the tuberculous, hypersensitive animals was marked. In the normal animals there was a slight infiltration of polymorphonuclear leucocytes which penetrated into the cornea from the capillaries of the limbus. The corneal cells themselves remained intact (Fig. 3). In the hypersensitive animals there was marked oedema of the cornea. The fibres were greatly swollen and were widely separated by fluid, and there was a dense migration of polymorphonuclear leucocytes into the cornea from the limbus. Many of the corneal cells were necrotic; others had undergone dissolution and had disappeared. Where the leucocytic infiltration was most intense no living corneal cells could be found, and here even the superficial layer of epithelial cells which covers the corneal fibres was necrotic. It was apparent that in the body sensitized through infection the cells of an avascular tissue are damaged and killed by contact with the bacterial protein (Figs. 1, 2 and 4). These results are in line with those of Holley (6), who observed ulceration of the cornea in tuberculous animals following the intracorneal injection of tuberculin; and they support *in vivo* our earlier *in vitro* tissue culture studies on bacterial hypersensitivity (1), which demonstrated that individual

tissue cells of the hypersensitive body are directly damaged and killed by contact with the specific protein, independently of any local vascular effects.

THE CORNEAL REACTION IN THE ARTHUS SENSITIZED ANIMAL

We then turned to the study of the reaction of the corneal cells in the Arthus type of hypersensitivity. For this purpose we used rabbits, because of their well known susceptibility to sensitization of the Arthus type. The animals were injected repeatedly intracutaneously and subcutaneously with 0.1 to 0.5 cc. of sterile foreign serum (dog or horse serum)[2] at four or five day intervals, until a state of hypersensitivity had been attained in which the intracutaneous injection of 0.1 cc. of the foreign serum would produce a large, necrotic reaction. A drop of the serum was then injected into the substance of the cornea of these hypersensitive animals. Macroscopically, the opacity produced by the bleb of fluid usually disappeared after a few hours, though occasionally a slight clouding persisted for a longer time. Microscopic study of the eyes removed at 24 and 48 hours after the injection showed that, in marked contrast to the destruction of the cells in the corneal tuberculin reaction, the corneal cells of the arthus sensitized animals were neither killed nor visibly damaged by contact with the specific protein. The only changes noted in the cornea were slight oedema, slight polymorphonuclear infiltration, and moderate swelling of the corneal fibers (Fig. 11). In the limbus at the corneal margin, however, where capillaries are normally present, inflammation was well marked—undoubtedly the result of the gradual spread of the injected serum to this region (Fig. 6). In these Arthus sensitized animals the slight alterations and lack of necrosis in the avascular cornea subjected to contact with the specific protein provided an impressive contrast to the necrotic lesions produced in the same animals when the protein was injected into the skin. Again confirming the results of the *in vitro* tissue culture studies mentioned above, these experiments demonstrated *in vivo* that in the Arthus type of hypersensitivity the individual cells of the tissues at large are not themselves sensitized in a manner that renders the specific protein

[2] Sterile horse serum, without preservative, was generously supplied by Dr. John F. Anderson, of E. R. Squibb and Sons.

capable of causing their death directly, as is the case in the hypersensitivity of infection.

THE REACTION OF THE VASCULARIZED CORNEA IN THE ARTHUS SENSITIZED ANIMAL

We next sought to determine whether it would be possible to demonstrate that in this type of hypersensitivity the capillaries are selectively sensitized. To this end we caused capillaries to grow into the cornea of one eye of Arthus sensitized rabbits, leaving the opposite eye normal. This can readily be done by injecting a minute drop of a suitable irritant into the center of the cornea. In the present experiments a heavy suspension of heat-killed tubercle bacilli was used as the irritant, as this had been found by Lewis and Montgomery (7) to be especially efficacious in causing vascularization of the cornea. Within a week to ten days after the injection of the bacilli a fringe of capillaries will have penetrated for a distance of several millimeters into the cornea from the scleral margin. When this vascularization had occurred, a small drop of the foreign serum to which the animal had been sensitized was injected into the cornea at a short distance from the capillaries, care being taken not to damage the capillaries mechanically; and a similar injection was made in the cornea of the opposite, non-vascularized eye, and into the vascularized cornea of normal control animals. Within the first day a marked opacity developed in the vascularized cornea of the Arthus sensitized animals, and haemorrhages made their appearance. In the opposite, non-vascularized eye the bleb was absorbed, leaving a clear or only slightly cloudy cornea. This was likewise the case in the vascularized corneas of the normal, non-hypersensitive control animals. It should further be stated that in these latter animals the injection of the serum into the vascularized cornea did not produce haemorrhage from the newly-formed capillaries. The haemorrhage observed in the vascularized corneas of the Arthus sensitized animals was, therefore, not due to trauma, but to the action of the foreign protein upon the sensitized vessels. This is made further evident by the fact that the haemorrhages appeared only after a lapse of time following the injection of the protein.

Microscopic study of the eyes removed at 24 and 48 hours after the intracorneal injection of the serum showed an intense inflammation

in the vascularized corneas of the Arthus sensitized animals, with swelling of the corneal fibers, oedema and marked polymorphonuclear infiltration, and also thrombosis and rupture of newly-formed capillaries with surrounding haemorrhage. In brief, the picture was that of a miniature Arthus reaction. An important difference between the reaction in the vascularized cornea and that in the skin, however, was the virtual absence of necrosis of tissue cells other than those of the capillaries. The corneal cells, as we have shown above, are not themselves hypersensitive; and since there is a tissue fluid circulation in the cornea which serves to nourish the corneal cells adequately, the cells are not dependent upon the newly-formed capillaries for their nutrition, and they are not appreciably affected by the destruction of those capillaries. In only a few minute areas immediately surrounding the damaged capillaries, where the tissue spaces were choked with haemorrhage and inflammatory exudate, was there a loss of some of the corneal cells; and, in contrast to the death of the superficial epithelium in the corneal tuberculin reaction, the epithelium was intact over even the most marked sites of reaction in the corneas of the Arthus sensitized animals (Figs. 5, 7, 8, 9, 10). The opposite, non-vascularized eyes showed the slight inflammation of the cornea described in the preceding section (Figs. 6 and 11). The vascularized corneas of the non-sensitized control animals exhibited only a slight inflammatory response to the injection of serum, as mentioned above.

DISCUSSION

These studies support the evidence provided by our previous tissue culture studies (1) that in bacterial hypersensitivity there exists a sensitization of tissue cells which causes their death on contact with the specific bacterial protein independently of any circulatory disturbance, while in the Arthus type of hypersensitivity no such sensitization of the extravascular tissue cells exists (2, 5). The present experiments show that in the Arthus sensitized body, contact of capillaries with the specific protein produces damage sufficient to cause rupture of the vessels even though the surrounding tissue cells are not detectably damaged. In the cornea, in which the nutrition of the cells is provided for by a fluid circulation and is not dependent upon the immediate presence of capillaries, the destruction of capillaries that have been induced to penetrate into the tissue does not entail

the death of the corneal cells. In other tissues, such as the skin, in which the nutrition of the cells is dependent upon the immediate presence of capillaries, destruction or obstruction of the vessels deprives the tissue of its nutrition and the tissue cells die. In the Arthus reaction as observed in the skin, haemorrhage resulting from destruction of the walls of capillaries, and occlusion of small vessels by thrombi, are prominent features. Since the *in vitro* tissue culture studies mentioned above, and the present *in vivo* observations, demonstrate that the extravascular tissue cells of the Arthus sensitized body are not killed by contact with the specific protein, their death in the Arthus reaction must be attributed to the interference with their nutrition that results from the vascular damage and from blocking of tissue spaces with haemorrhage and exudate. Gerlach (9) has called attention to the compression of capillaries caused by the distension of the tissue spaces with exudate and by the great swelling of the collagenous fibers in the Arthus reaction, and this factor may add to the ischaemia caused by vascular rupture and thrombosis.

Opie (10), while appreciating fully the vascular damage that occurs in the Arthus reaction, concluded that the extravascular tissue damage is due in part to a direct toxic effect of the precipitate which forms when the specific protein and its precipitin meet in the tissue spaces. He attempted to demonstrate that the precipitate is toxic by mixing the protein with the precipitating serum of the sensitized body and injecting the resulting precipitate into the skin of normal animals. The precipitate produced some degree of inflammation and attracted polymorphonuclear leucocytes, but, in spite of the fact that a large amount of precipitate was brought into direct contact with the tissues, the inflammation was always much less than that which occurs in the Arthus reaction and, important to note, no necrosis was ever produced. In the tissue culture experiments of Aronson (4) and ourselves (5), the cells of the Arthus sensitized body that were planted in mixtures of the animal's serum with the protein to which it had been sensitized were unharmed by the abundant specific precipitate which formed in those mixtures. In cultures in which the cells of Arthus sensitized animals were grown in mixtures of the foreign protein with the serum of normal animals, no precipitate formed, and the cells were unharmed by contact with the unprecipitated protein. These observations, together with the failure of the specific foreign protein to kill the

corneal cells of Arthus sensitized animals in the present experiments, makes it clear that the necrosis of the extravascular tissues in the Arthus reaction cannot be ascribed to a direct toxic action of either the foreign protein or its specific precipitate.

In the Arthus sensitized body, therefore, the sensitivity that determines necrosis appears to be limited to the blood vessels, and especially to the endothelium. The procedure required for the establishment of Arthus sensitivity (i.e., parenteral injection of foreign protein) entails the development of anaphylactic sensitivity, in which involuntary muscle, including that of the blood vessels, is sensitized in a manner which causes it to contract spasmodically on contact with the specific protein. Abell and Schenck (20), by means of the transparent moat-chamber in the rabbit's ear, have observed directly the occurrence of temporary arteriolar spasm when foreign serum was introduced into the chamber in sensitized animals. Arteriolar spasm, therefore, may contribute to the ischaemia at the site of the Arthus reaction. Endothelial damage, however, appears to be a more important factor in the production of the ischaemia. That the capillary endothelium of the sensitized body is damaged by contact with the specific protein is clear both from the increase in its permeability, as manifested by the prompt and marked local oedema that occurs following the injection of even high dilutions of the protein, and from haemorrhage which results from rupture of necrotic capillaries in the more severe reactions produced by more concentrated solutions of the protein.

In the literature there are several observations on corneal hypersensitivity upon which the present studies bear. In 1911, Wessely (21) noted that if foreign serum were injected into the cornea, the bleb was absorbed leaving a clear cornea, but 12 to 14 days later a decided clouding of the cornea occurred. This was confirmed by v. Szily (22), v. Hofe and Krantz (23) and others. v. Poppen (24) and Julianelle, Morris and Harrison (25) noted that if foreign protein were injected into the cornea of one eye either once or repeatedly, the reinjection of the protein into that cornea at a later period produced a much more marked prompt clouding than did a simultaneous injection into the opposite untreated eye. These observations have been interpreted as indicating that a local injection of foreign protein into the cornea sensitizes that cornea to a higher degree than the tissues elsewhere. The experiments reported in the present paper provide a

different interpretation. Corneal clouding in the above experiments is the result of the escape of a fluid and cellular exudate into the substance of the cornea, and this exudate is derived from the blood vessels. The presence of capillaries in the cornea, or of increased vascularity at the limbus, will obviously provide a source from which exudate may be derived more promptly in larger amount than in the case of the non-vascularized cornea. The present experiments show that in animals sensitized by injecting the foreign protein into sites far removed from the cornea, a marked corneal clouding will result promptly from the injection of the protein into the cornea if the latter has first been vascularized, and will not occur, regardless of how great a degree of generalized Arthus type sensitization is present, if the cornea has not been first subjected to procedures which increase its vascularity. The essential factor for the prompt production of marked corneal reactions in the Arthus sensitized body, therefore, appears to be simply an increased corneal vascularity, rather than any postulated higher degree of local hypersensitivity of the cornea itself; and, in fact, while the above-mentioned investigators did not correlate the occurrence of the marked corneal reactions with vascularization of the cornea, a careful examination of their protocols discloses the fact that vascularization was present at the time the marked reactions occurred. A single intracorneal injection of foreign serum can incite corneal vascularization, and repeated injections invariably do so. That vascularization has occurred at the time when the cornea clouds in the Wessely experiment (i.e., 10 to 14 days following a single injection of foreign serum into the cornea) can be plainly seen in the illustrations in v. Szily's monograph (22 b).

SUMMARY

In the Arthus type of hypersensitivity there is vascular sensitivity but the cells of the tissues at large are not themselves sensitized. Tissue death in the Arthus reaction results primarily from impairment of nutrition due to vascular damage and to clogging of the tissue spaces with exudate and haemorrhage.

It has been shown by numerous investigators that if foreign protein is injected one or more times into the cornea of one eye, a subsequent injection after some days leads to a more marked corneal reaction than occurs as a result of the injection of the protein into the opposite,

untreated eye. This has been attributed to the development of local hypersensitivity in the treated eye. The present experiments indicate that the more marked reaction in the treated eye is due not to a higher degree of local sensitization of the corneal tissue, but to the increased vascularity which results from the preliminary intracorneal injections, and from which a greater amount of exudate can be derived more promptly.

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PLATE I

(Microphotographs by Mr. Milton Kougl)

FIG. 1. Cornea of tuberculous, hypersensitive animal (GP-39) 48 hours after intracorneal injection of tuberculoprotein. The dark areas are sites of marked inflammatory infiltration and necrosis. The cornea is greatly thickened by the exudate and by swelling of the corneal fibers (see Fig. 4).

FIG. 2. High power field of site of reaction in Fig. 1. Intense infiltration of inflammatory cells, practically all of which are necrotic. No living corneal cells present.

FIG. 3. Cornea of normal animal (GP-45) 48 hours after intracorneal injection of tuberculoprotein. Fibers are separated by fluid; a few inflammatory cells are present. The numerous very flat, elongated nuclei of the corneal cells are normal in appearance. There is no necrosis.

FIG. 4. Cornea of eye shown in Fig. 1, outside the zone of marked inflammation. There is pronounced oedema and swelling of the corneal fibers. A few inflammatory cells are present. No intact corneal cells are seen. Compare with similar site in corneal Arthus reaction as shown in Fig. 9.

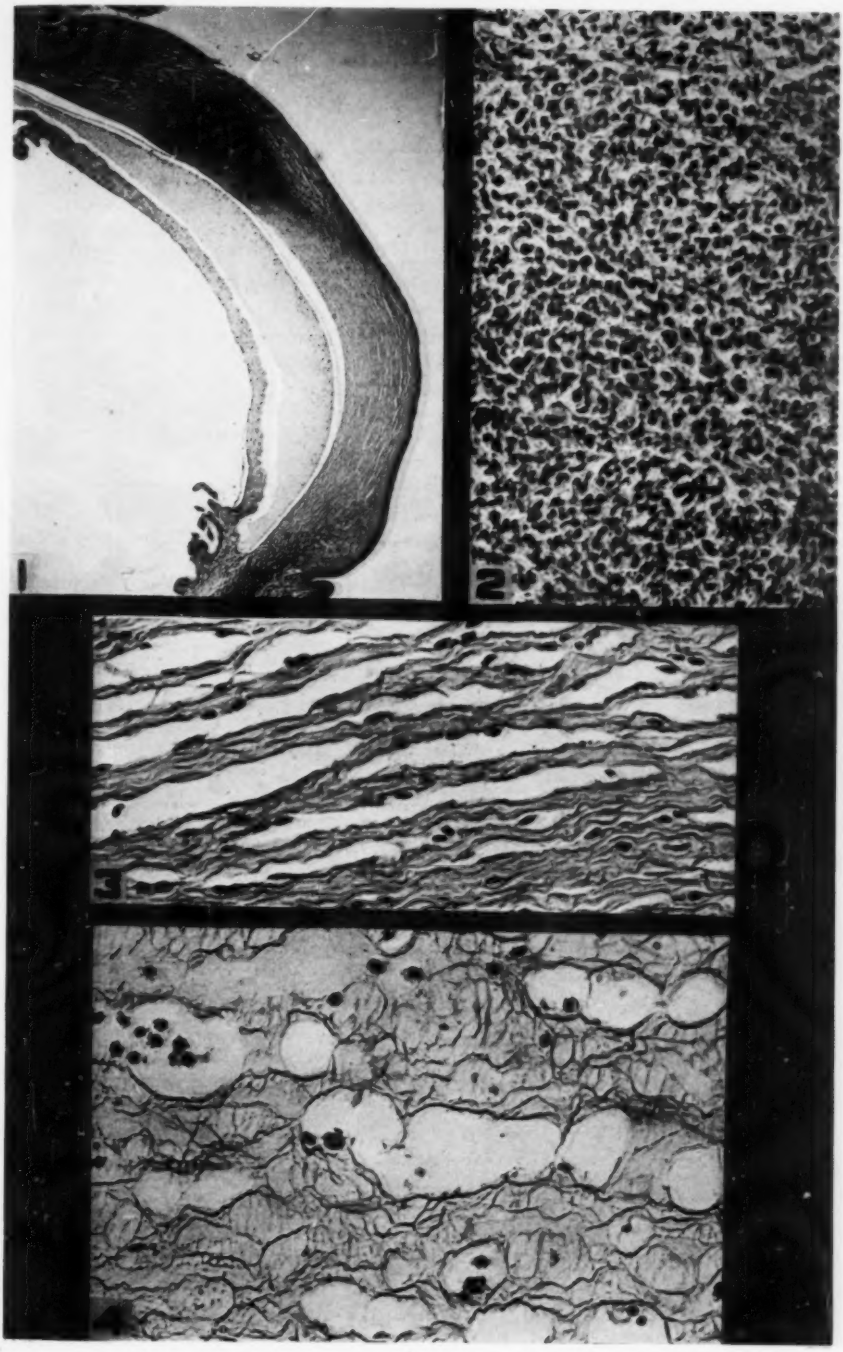
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PLATE II

FIG. 5. Cornea of Arthus sensitized animal (R-8) 48 hours after intracorneai injection of the specific serum. This cornea had been vascularized before the test injection of the serum. The dark areas are sites of marked inflammation and haemorrhage. Control vascularized corneas examined at the same period following serum injection show no such haemorrhage and inflammatory infiltration. The vascularization in this cornea extended from the limbus to about the point marked "A." The zone occupied by the test injection of serum was approximately that indicated by "B." The thickening of the cornea by exudate and by swelling of the fibers is clear from a comparison with the more normal thickness shown at the top of the photograph, and with that of the cornea in Fig. 6.

FIG. 6. Cornea of opposite eye of rabbit of Fig. 5. This cornea was injected with the specific serum and fixed at the same time as that in Fig. 5, but it was not vascularized. Inflammation can be seen about the capillaries that are normally present at the limbus, and inflammatory cells extend for a short distance into the cornea. The test injection of serum was made at approximately the same level as that in Fig. 5.

FIG. 7. Field from cornea of Fig. 5, showing inflammatory exudate and haemorrhage separating the corneal fibers in the vascularized zone. Most of the corneal cells are intact, and the overlying epithelium is normal.

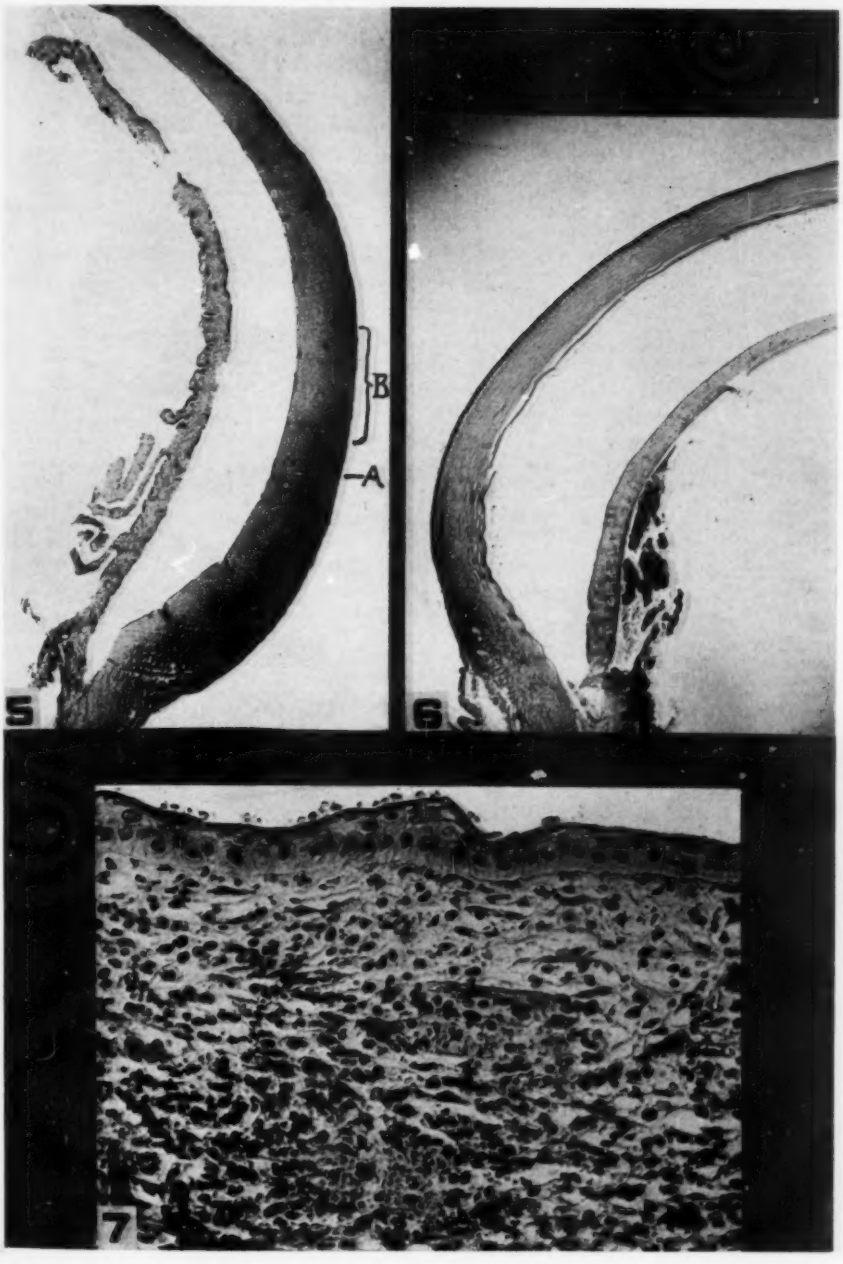
PLATE III

FIG. 8. Field from cornea of Fig. 5, outside the vascularized zone. Note the intact, elongated corneal cells separated by the exudate.

FIG. 9. Field from cornea of Fig. 5, still further removed from the zone of intense inflammation than is the field in Fig. 8. There is marked oedema and swelling of the corneal fibers, and a few leucocytes are present. The corneal cells are intact. Compare with similar zone in the tuberculin reaction, as shown in Fig. 4, in which the corneal cells have disappeared. Compare the swollen fibers with the approximately normal ones in Fig. 3.

FIG. 10. Field from vascularized zone of cornea of Fig. 5, showing one of the newly-formed capillaries at the site of the reaction. Part of the capillary wall is necrotic, and the vessel contains a thrombus in which there are many necrotic leucocytes.

FIG. 11. Cornea of Arthus sensitized rabbit (R7L) 48 hours after intracorneal injection of the specific serum. This cornea had not been vascularized before the test injection. There is oedema, swelling of the corneal fibers, and infiltration with inflammatory cells, but the corneal cells (elongated nuclei) are not necrotic.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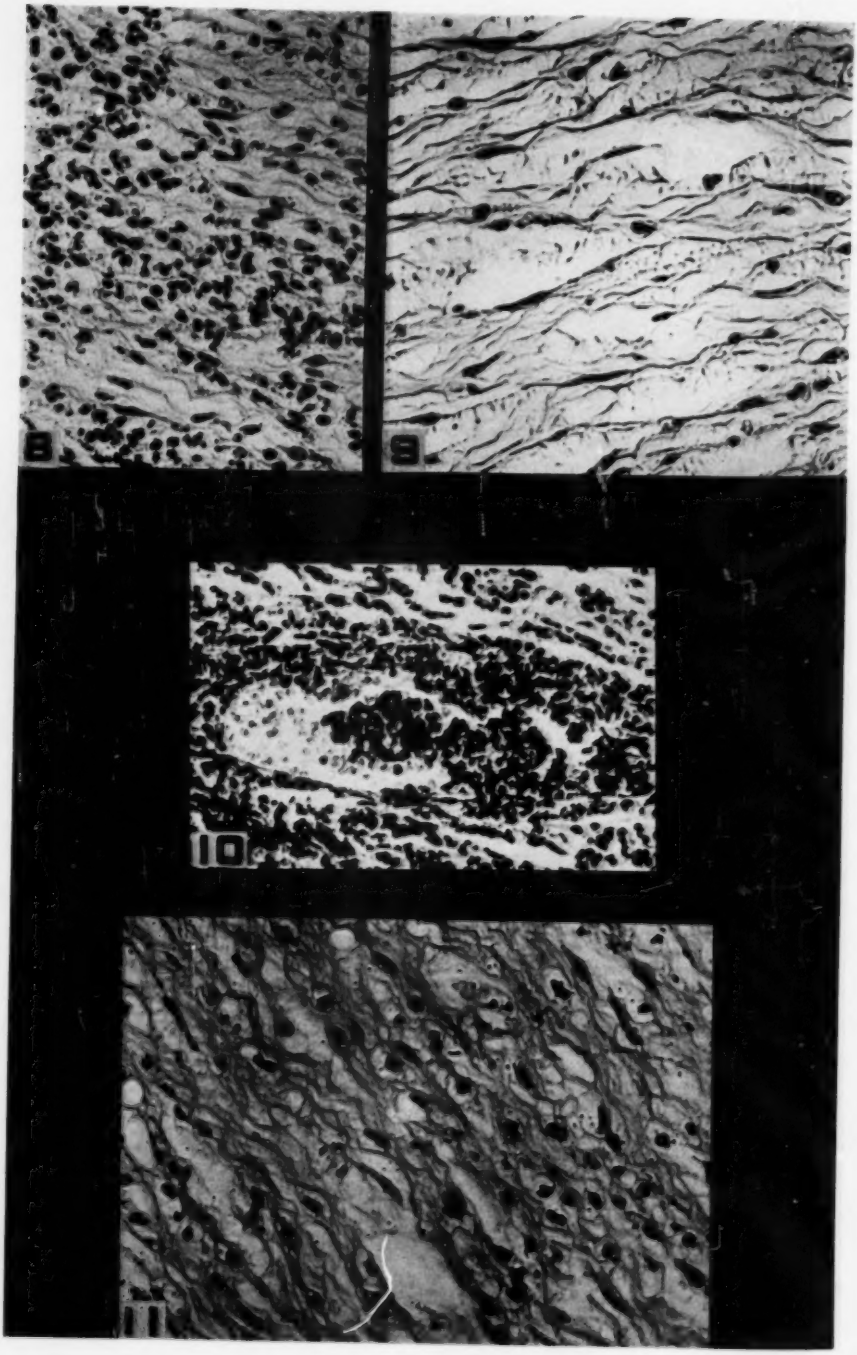
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THE EASTERN VARIETY OF ROCKY MOUNTAIN SPOTTED FEVER

The Experience on the Adult Medical Service of The Johns Hopkins Hospital, Including the Report of a Fatal Case Showing Thrombocytopenia

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Although it is now over nine years since the eastern variety of Rocky Mountain Spotted Fever was first recognized (5) and there are several excellent descriptions of the disease (1, 2, 4, 8), only six cases have been so diagnosed on the adult medical service of The Johns Hopkins Hospital. Because one of the most recent cases presented a strikingly typical clinical and autopsy picture of the disease with the unusual feature of thrombocytopenia, it is reported in detail. Then because of their rarity, all six cases are analysed from the point of view of history, physical and laboratory findings.

CASE REPORT

D. C. B., No. 170752, a farmer 52 years of age from Belair, in northeastern Maryland, was admitted to the medical service on June 8, 1939 because of fever and a rash.

His family and past histories were non-contributory.

The exact onset of the present illness was difficult to date. Nine days before admission the patient had complained of mild pain in his right elbow, anorexia, weakness and headache. Seven days before admission his wife noticed a rash about his ankles and thought he might have had some fever. The next day he had chilly sensations, but no rigor, vague pains in his legs, several nose bleeds and three to four loose stools. The day before admission to the hospital he was delirious. The morning of admission a rash was observed on his trunk.

Although neither the patient nor his family could recall any specific tick bites, they did volunteer the information that he often found ticks on his body and clothes.

On admission to the hospital the patient was acutely ill. He was lying quietly,

flat in bed, breathing somewhat rapidly but without difficulty. His temperature was 104°F., pulse 120 per minute and respirations 40 per minute. He was a red faced, light complexioned man who looked younger than his stated age. On the skin there were multiple pin-point and pin-head sized macular areas and a number of maculo-papular erythematous lesions, a few of which were hemorrhagic. These were diffusely and symmetrically distributed over the body except for the head, palms of the hands and soles of the feet where none were found. They were most profuse about the ankles, buttocks, axillae and shoulders. The lesions were apparently not painful nor pruritic. The conjunctival vessels were engorged.

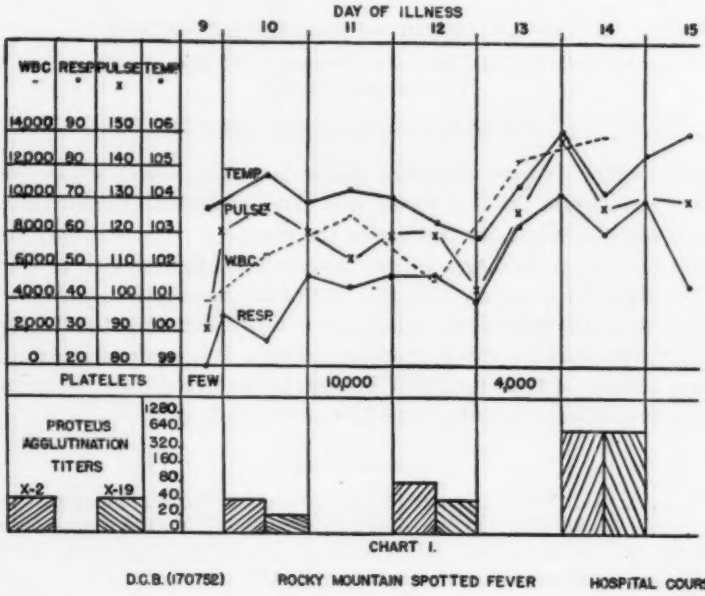

CHART I.

D.G.B. (170752) ROCKY MOUNTAIN SPOTTED FEVER HOSPITAL COURSE

There were crusts of old blood about the nostrils. The teeth were carious, pyorrhea was advanced and the tongue was dry. The mucous membranes were of good color. The lungs were clear to percussion and auscultation. The heart was essentially normal with a regular rhythm though somewhat rapid rate. The blood pressure was 115/75. The abdomen was soft. The spleen was not felt, but the liver edge could be percussed two finger-breadths below the costal margin. There was no generalized lymphadenopathy. Neurological examination showed no abnormalities except very sluggish deep tendon reflexes.

On admission to the hospital laboratory studies revealed a red blood cell count of 4.5 per mm^3 and a white blood cell count of 3,900 per mm^3. The differential

count (Wright's stain) was 55 per cent juvenile polymorphonuclears, 28 per cent adult polymorphonuclears, 9 per cent lymphocytes and 8 per cent monocytes. There were a few platelets in the smear. The tourniquet test was positive (7). The icteric index was 5 units. The urine was alkaline and contained albumin (plus 3) as well as 10-20 white blood cells per high-power-field of a centrifuged voided specimen. The blood non-protein nitrogen was 38 mgm. per 100 cc. The CO_2 combining power of the plasma was 56 vol. per cent and the plasma chloride content was 86.4 m.eq. per liter. Agglutinations against Proteus X-2 and X-19 were negative. The blood culture was sterile.

FIG. 1. SCROTAL NECROSIS. (D.C.B. #170752. DAY 13)

The patient was on the ward seven days. Temperature, pulse, respirations, agglutination tests and blood studies are indicated in Chart 1. During the first few days he was occasionally restless, and several times got out of bed, though most of the time he was very drowsy. After the tenth day (admitted on the ninth day of his illness) he was irrational, rarely oriented and progressively less responsive. He was incontinent. Frequent coarse tremors of the hands were noticeable throughout his illness. On the tenth day stiffness of the neck became apparent. On the eleventh day slight ankle edema was noted. Terminally, on the fifteenth day, moist rales were heard at both lung bases.

The development of the skin lesions was one the most striking features of his clinical course. Shortly after admission they became more pronounced especially over the abdomen and the dorsa of the feet. They also became more purpuric and in many places on the extremities, confluent. They also appeared on the soles of both feet. On Day 13 scrotal, penile and sacral necroses were noted (Fig. 1). The skin in general became more dusky and on the morning of Day 15, necrosis was also observed over the right elbow and heel. Biopsy of the skin taken on Day 12 was reported by Dr. J. McManus of the Department of Pathology as showing subcutaneous perivascular lesions such as are seen in Tick Bite Fever (3).

The white blood cell count as indicated on the chart rapidly increased. There was a marked shift in the differential so that on Day 14 when the leukocyte count was 13,840 per mm^3 there were 19 per cent juvenile polymorphonuclears, 70 per cent adult polymorphonuclears, 10 per cent lymphocytes and 1 per cent monocytes. Platelet counts (6) made on Days 11 and 14 by the indirect method showed only 10,000 per mm^3 and 4,000 per mm^3 respectively.

Shortly before death, on the fifteenth day of his illness, respirations became labored, especially expiration, and the blood pressure which for the few days before had been about 100/80 fell to 58/40. The patient appeared to die as the result of circulatory failure.

Treatment was entirely symptomatic and included daily intravenous administration of glucose and saline, as well as two small transfusions on the thirteenth and fourteenth days.

Autopsy (# 16445) was performed four hours after death. The skin was still most remarkable. Although the very extensive papular erythematous lesions seen during life had faded considerably, the body was still diffusely covered with a purplish hemorrhagic rash which was of a more intense purplish-blue in the dependent parts. The lungs were slightly emphysematous. On their pleural surfaces there were a few scattered purplish areas and in both lungs there were a few areas of consolidation which because of their location were thought to be due to aspiration pneumonia. There were a few hemorrhages beneath the tracheal mucosa. The spleen weighed 310 grams. Its capsule was stretched and there were four wedge-shaped infarcts, two of which were pale and whitish. Neither the malpighian bodies nor the trabeculae were prominent, but the splenic pulp was dark purplish-red, soft, mushy and bulging. The mucosa of the epiglottis was reddened and covered with whitish gray exudate with a few areas of ulceration. The lining of the urinary bladder was roughened and engorged. There were a few hemorrhages beneath the rectal mucosa. The meninges were normal but the brain was edematous and its vessels were engorged. The cerebral convolutions were slightly flattened. The femur marrow was a deep red.

Microscopic Examination: The typical lesion involved the small arterioles and capillaries. It was characterized by a swelling and proliferation of the vascular endothelium and a perivascular accumulation of mononuclear cells, some of which

resembled plasma cells; others, large lymphocytes and macrophages. Occasionally a thrombus was found attached to the wall of a small vessel about which there were often small foci of necrosis. These lesions were most abundant in the scrotal skin, myocardium, brain, choroid of the eyes, pharynx, kidneys, spleen and liver (Figs. 2 and 3).

In the heart the muscle bundles in places were so extensively infiltrated with mononuclear cells that the picture resembled that of myocarditis. The endothelial surface was not greatly altered. The lungs showed extensive pulmonary edema and a considerable thickening of the alveolar septae as the result of infiltration of mononuclear cells. The spleen did not show evidence of the typical acute splenic tumor but many anemic infarcts were seen in addition to the typical perivascular lesions. The most notable changes in the liver were the mononuclear infiltrations about the periportal blood vessels. The renal glomeruli were not greatly altered but the tubular epithelium was swollen. Sections through the duodenum, ileum and rectum showed the typical vascular lesions in the submucosa. In the skin the lesions were most evident in the corium. Occasionally a mural thrombus was found in one of the smaller blood vessels, with associated small areas of hemorrhage. Sections through the skin of the scrotum showed in many places necrotic epithelial cells. The vessels of the submucosa were engorged and there were extensive hemorrhages into the layers just beneath the epithelium. Many mononuclear cells were found about the capillaries. The lumina of many capillaries were occluded by these cells. The loose areolar tissue had the appearance associated with edema. In the sections of brain and spinal cord there were many small perivascular accumulations of mononuclear cells with areas of edema and pyknotic nuclei. These lesions were especially prominent in the cerebellum. The femur marrow was hyperplastic as the result of an increase in the number of cells of both myeloid and erythroblastic series. There was a normal number of megalokaryocytes in the marrow of both the femur and the vertebrae.

Rickettsia bodies were found in large numbers in the myocardium, testes, scrotal skin, skin of the upper extremity, wall of the pharynx and occasionally in the cerebral cortex. They appeared chiefly in the cytoplasm of the mononuclear cells in the lesions already described. These cells stained a deep purplish-red with the Giemsa technique and could be distinguished with the low power of the microscope. When examined under the high power, the cells were found to be filled with densely packed purplish-red granules (Figs. 4 & 5). In contrast these bodies were much less frequently found in the vascular endothelium. None were seen in the Kupffer cells of the liver.

Comment: The positive tourniquet test noted when routine vena puncture was done on admission, aroused interest in the number of platelets in the circulating blood. As already reported, these proved to be reduced in number. The fact that the bone marrow showed an

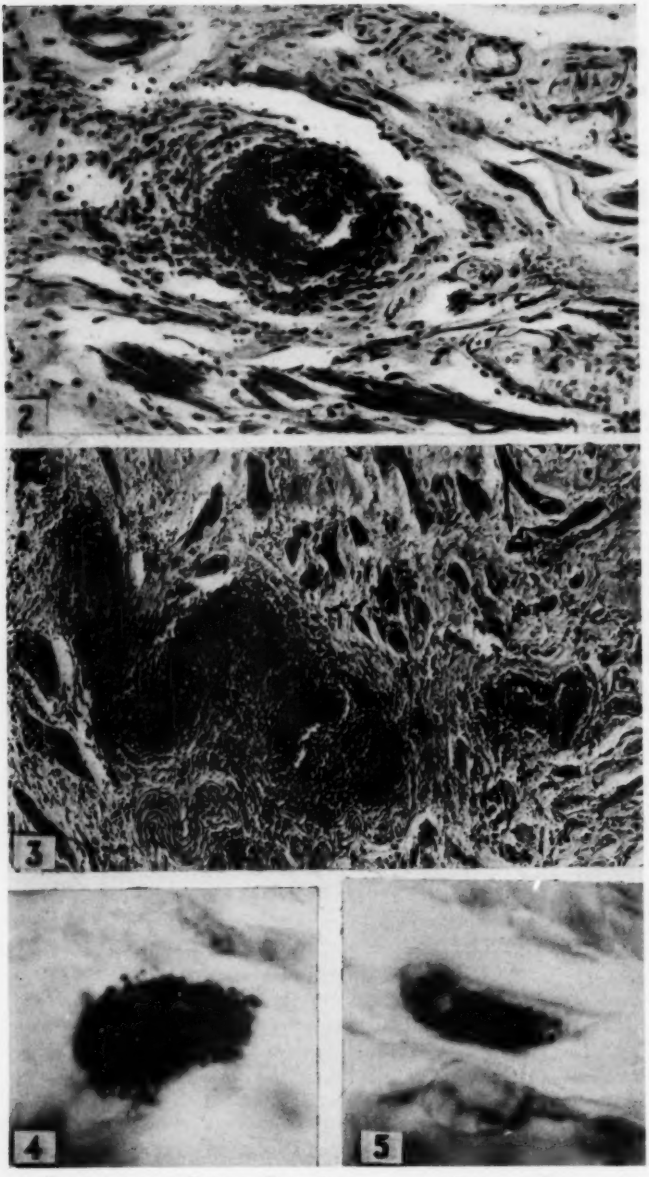

FIG. 2. PERIVASCULAR ROUND CELL INFILTRATION SCROTAL SKIN. 200 ×

FIG. 3. PERIVASCULAR ROUND CELL INFILTRATION SCROTAL SKIN. 100 ×

FIG. 4. RICKETTSIA BODIES IN MONONUCLEAR CELL OF THE SCROTAL SKIN. 1800 × (OIL) GIEMSA STAIN

FIG. 5. RICKETTSIA BODIES IN MONONUCLEAR CELL IN HEART MUSCLE. 1800 × (OIL) GIEMSA STAIN

apparently normal number of megalokaryocytes is not inconsistent since this has also been reported in thrombocytopenic purpura.

A search of the literature failed to reveal any mention of thrombocytopenia or of the finding of a positive tourniquet test in Rocky Mountain Spotted Fever. The note is made that the rash may sometimes resemble that seen in thrombocytopenic purpura (8). It has been observed (1) that the petechial character of the rash may be accentuated by the application of a tourniquet. We have since had the opportunity of observing another patient (J. S. #170773) with this disease in whom the tourniquet test was also positive on the day his rash appeared. At that time in this patient the platelets were 120,000 per mm^3 by direct, and 160,000 per mm^3 by indirect count.

A positive tourniquet test has long been recognized in scarlet fever. Similar observations have been reported in poliomyelitis and we have on several occasions seen patients with pneumococcal pneumonia in whom application of a tourniquet even for a short while would produce a crop of petechiae. Therefore this reaction in a febrile disease is apparently not specific. However, the thrombocytopenia discovered in this case seemed to be of sufficient interest to record. It may have been one of the factors responsible for the markedly hemorrhagic nature of the skin lesions, especially late in the disease when the platelets were so markedly decreased.

ANALYSIS OF CASES

On the adult medical service of The Johns Hopkins Hospital there have been 6 patients diagnosed clinically or at autopsy as Rocky Mountain Spotted Fever. There was one in each of the years 1932, 1934, 1937, 1938, and two in 1939. All but one appeared in the month of June; the exception occurred in August. All six of the patients lived or worked in the country and four were aware of exposure to ticks. Only one remembered a definite bite. Five were male (3 white and 2 colored), and one was a white female. Their ages varied from 12 to 82. Three patients died.

The symptoms in all patients were similar, and included headache, malaise, anorexia, fever, chilly sensations and aching pains in muscles and joints. Two patients, both of whom died, had predominantly gastro-intestinal symptoms, chiefly vomiting and diarrhea: one was

admitted with a diagnosis of gastro-enteritis, and the other was suspected of having an intestinal obstruction. One patient was admitted on the 3rd day after the onset of symptoms; three came into the hospital on the 7th day, one on the 9th and one on the 15th day after the onset of symptoms.

In all but the one patient admitted on the 15th day the temperature was as high as 104°F. and in the three fatal instances the temperature remained elevated, rising before death as high as 106°. In the other three patients the temperature fell by lysis to essentially normal levels by the end of the 3rd week. The pulse in the three recovered patients varied between 60 and 128 per minute, while in the three who died the range was from 90 to 160 per minute. Respirations in the patients who recovered were essentially normal, in the others they were very shallow and rapid, especially during the last few days, varying between 40 and 72 per minute with a mean at about 52 per minute. Despite this, in the two patients in whom it was determined, the plasma CO_2 combining power remained between 42 and 56 volumes per cent.

In only three instances could the day of appearance of the rash be ascertained. In two it appeared on the third day, and in one on the tenth day after onset of symptoms. The rash which was most profuse over the extremities was characteristically erythematous and maculopapular with some purpuric lesions in all four of the white patients. One colored man showed only a few macular red spots over his abdomen and on his buccal membranes. In the case of the other colored man no rash was found. In only the one case, described in detail above, did the involved skin become necrotic.

In all but one instance a definite conjunctivitis was noted. In three, crusts of old blood were found in the nostrils. Only one patient showed jaundice. He died 26 hours after admission. In all, examination of the heart and lungs was essentially normal except for some rales terminally in two of the patients who died. The blood pressure in all the patients on admission was normal, though in two there was a gradual fall before death. In none were the peripheral blood vessels especially remarkable. The spleen was palpable in only two cases, and in only one was there generalized lymphadenopathy. In two

of the patients there was some slight pitting edema of the ankles before death.

All, with the exception of the patient admitted on the 15th day of her illness, were drowsy on admission. The three patients who died became irrational, and finally stuporous. Three had meningismus with hypoactive deep tendon reflexes. In two of these, spinal punctures were done but the spinal fluid was found to be normal. In two fatal cases the patients had coarse tremors of the hands.

Laboratory studies showed essentially normal urines with occasional slight albuminuria and pyuria. Blood examinations revealed red blood cell counts from 3.5 to 5.3 million per mm^3, with the hemoglobin between 11.3 and 15.6 gms. The white blood cell counts were usually lowest on admission varying from 2,400 to 15,700 per mm^3 and rising during the height of the disease to as high as 41,000 per mm^3. The differential leukocyte count showed a great preponderance of polymorphonuclear leukocytes. Occasionally as high as 50 per cent of the white blood cells were juvenile polymorphonuclears. The platelets in four of the instances, as judged by the appearances of the blood smears, were normal in number; but in the last two patients who were observed personally they were found to be reduced. In one they were only moderately reduced, but in the other instance, which has been reported above, they were as low as 4,000 per mm^3. In both of these patients the tourniquet tests were positive.

Weil-Felix agglutinations were done in all but the case seen in 1932 and all except one, at sometime during their hospital stay, showed agglutination titres of over 1/320 with either Proteus X-2 or X-19. No definite differential value could be attached to the X-2 strain as compared with the X-19 strain.

The three patients who recovered remained in the hospital 18 to 20 days. The three patients who died did so on the 7th, 13th, and 15th days of their illness, respectively.

Autopsies were performed in all three fatal cases, which included the one in which the Weil-Felix agglutination had not been done and the one in which there had not been any positive agglutinations. All three patients showed many scattered characteristic vascular

lesions similar to those described above. In each of these three cases Rickettsia bodies were demonstrated.

COMMENT

It is generally stated that this disease is more frequent in children, but more severe in adults. The experience at The Johns Hopkins Hospital tends to bear this out. Although there were only six cases on the adult medical service with a 50 per cent mortality; on the pediatric service, during this same period, there were fifteen instances of the disease with a mortality of 13.3 per cent. In all there were twenty-one cases in the hospital records and five of these (23.8 per cent) died. This figure agrees quite closely with the statement of Dyer (quoted in (2)) that the mortality of Rocky Mountain Spotted Fever in the East is about 25 per cent.

The symptomatology presented in our analysis of the six adult case histories is essentially similar to that described by Parker (4). However, there were a few findings that are not generally stressed and which were rather striking in our review. These were the very rapid, shallow respirations in the fatal instances without the presence of advanced pneumonia, the prominent gastro-intestinal complaints of two of our patients, the fact that in only two patients was the spleen palpable and that in only one was there generalized lymphadenopathy.

Lillie (3) in attempting to differentiate the pathology of the Eastern from the Western type of this disease, pointed out the prominence of bronchopneumonia and brain lesions in the former, and their infrequency in the latter type. It is therefore of some interest that only one of our patients had bronchopneumonia. In the two cases in which the brain was examined microscopically, typical lesions were demonstrated. He also mentioned the fact that scrotal necrosis, such as seen in the case reported above, had not yet been described in the eastern variety of Rocky Mountain Spotted Fever in man. It would seem that severity of infection, rather than geography or vector, is of importance in determining the pathological picture.

SUMMARY

1. During the past nine years there have been six cases of Rocky Mountain Spotted Fever on the adult medical service of The Johns Hopkins Hospital.

2. Three of these were fatal.

3. One of these because of its characteristic picture and the unusual feature of thrombocytopenia, is presented in detail with autopsy notes.

4. The six cases are analyzed from the point of view of history, physical and laboratory findings.

The authors wish to acknowledge the encouragement and permission to publish these cases, given by Drs. W. T. Longcope and W. G. MacCallum. They are also grateful to Drs. M. M. Wintrobe and L. W. Eichna for many helpful suggestions in the preparation of this report.

The clinical photograph was made by Mr. Mahon and the microphotographs by Mr. Milton Kougl.

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BOOK REVIEWS

(The reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Menstrual Disorders: Pathology, Diagnosis and Treatment. By C. FREDERICK FLUHMANN. 329 pp. Illus. $5.00. *W. B. Saunders Company, Philadelphia, 1939.*

The subject matter covered by this book is of fundamental importance to the gynecologist, for one might say that he who has a thorough understanding of the physiological principles underlying menstruation, and of the pathological aberations of this phenomenon, has acquired the ground work necessary for scientific gynecology. The author has presented this material in a thorough, but concise and readable volume, which should be of value not only to the gynecologist and obstetrician, but to physicians in other fields, particularly the general practitioner and internist.

The introductory chapter, dealing historically with ancient and former concepts of the menstrual phenomenon, serves to explain many of the superstitious and erroneous ideas which still persist. The treatment of the physiological and endocrinological aspects of the subject has been confined, as largely as possible, to scientifically proven ground, and the temptation, often so enticing when dealing with this subject, to wander into speculative fields has been largely avoided.

In dealing with diagnosis the author has not neglected to set forth repeatedly the warning, that serious organic lesions must be ruled out before abnormal uterine bleeding can be assigned to that group known as functional. This warning is particularly appropriate at the moment, when the tendency to attribute many ills to endocrine disturbances is so prevalent.

The suggested therapy is along conservative lines, and particularly as regards endocrine therapy, only those measures that are of proven value are recommended.

The literature has been referred to frequently and the bibliography alone should be quite valuable.

H. S. E.

Clinical Laboratory Methods and Diagnosis. By R. B. H. GRADWOHL. 2nd ed 1607 pp. Illus. $12.50. *C. V. Mosby Company, St. Louis, 1938.*

This work, already very big in its first edition, is now half again as large. There are numerous colored plates and photographs, some very good, others, especially the plates of leucocytes, rather poor. The section on parasitology particularly has been extended, with noteworthy improvement.

It may be asked whether one would like to have in a book on laboratory technique an unwieldy compilation of a great variety of procedures (the book weighs over 8 pounds) accumulated without critical selection, or a well digested, accurate account of the best techniques with only brief mention and references to other methods. The reviewer prefers the latter type of book.

Surely the statement on page 300, that the normal red cell count in the adult male is between 5 million and 5 million one hundred thousand cells, is a typographical error. One looks for a more complete statement on normal blood values in vain.

M. M. W.

Tissues of the Body. By W. E. Le Gros Clark. 372 pp. Illus. $5.50. *Oxford University Press, London, New York, etc., 1939.*

This book is an outgrowth of lectures presented to beginning students in Anatomy by the professor of Anatomy at Oxford. Writing in a lucid and engaging style, the author has succeeded in producing a volume that is unique in the way of introductory anatomical texts. In no sense is it a handbook of either gross or microscopical anatomy, although in actuality it serves to bridge, for the beginner, the too often apparent gap between these two divisions of the anatomical curriculum.

The outstanding feature of the book is its broad outlook, as would be expected by those who know the author. The whole of Anatomy is presented as a dynamic science that deals with the "fundamental principles of the growth, differentiation and structural adaptation of living tissues". In a way the book is a measure of the advance in anatomical thinking and teaching since the turn of the century. For it may be predicted that there are few anatomists left who will question the importance to the student of being exposed to this dynamic point of view, even though it must be attained, to use the author's own words, "at the expense of some superfluous knowledge of the topographical minutiae of human anatomy".

W. L. S.

Health at Fifty. Edited by William H. Robey. 299 pp. $3.00. *Harvard University Press, Cambridge, Mass., 1939.*

Annually since 1907 the Faculty of the Medical School of Harvard University has sponsored a series of free Sunday afternoon lectures making available to the public the current knowledge of the causation, treatment, and prevention of disease. Twelve of these lectures most of which deal with subjects of interest to the mature adult have been collected in book form. There are chapters, for instance, *Heart Disease* by Blumgart; *Blood Pressure* by Soma Weiss; *Rheumatism* by Bauer; *Vitamins* by Wolbach; *Mental Health* by Macfie Campbell. This book can well be read by physician and layman alike. A medical teacher will obtain many valuable hints from these simple but graphic presentations. To the specialist, of course, each chapter is inadequate and occasionally inaccurate.

To the physician not particularly versed in one of the specialties, however, the simple popular presentation serves to clarify many fundamental points.

This little book is further evidence of the active interest the Medical School of Harvard University takes in the popular demand for up-to-date information on medical topics. These lectures afford an important opportunity to educate the public properly and to expose fads, quacks and charlatans. Such friendly efforts in behalf of the laity on the part of distinguished and dignified members of the medical profession, are conspicious by their small numbers. We are heartily in sympathy with this whole program.

H. M. T., Jr.

Malaria in Panama. By JAMES STEVENS SIMMONS AND OTHERS. 326 pp. $1.10. *The Johns Hopkins Press, Baltimore, 1939* (American Journal of Hygiene Monographic Series, No. 13, January, 1939).

The importance of the Panama Canal to the United States makes essential healthy living conditions for civilian employees of the Canal, the military and naval forces, and also for the natives of the Republic of Panama who live in the vicinity of the Canal Zone. During the years of construction malaria was the most important problem that faced the men responsible for the health of the Canal personnel, and although the incidence now is remarkably low, constant vigilance is still necessary. In this 326-page book, composed of eleven chapters grouped into three parts, and a resume, bibliography, and index, the authors review the past accomplishments in the battle against malaria in Panama; they outline control measures and the present status of the disease, with particular reference to its prevalence among U. S. army troops, and discuss problems that have arisen as a result of changing conditions.

Control measures have brought down the incidence of malaria among Canal Zone employees to a very low level, but only small areas have been sanitated, while as yet nothing can be done about the mosquitoes breeding in Gatun Lake, and in jungle streams, pools, and swamps. Furthermore, in the Canal Zone there are a number of negro farmers, who, in order to relieve distress caused by unemployment following completion of the Canal, were allowed to settle in unsanitated parts of the Zone. Among these settlers are many malaria carriers, who constitute a menace to the other inhabitants of the Canal Zone. Another source of infection are the carriers in the large native population of the areas bordering the Canal Zone, and improved transportation facilities between the Zone and outlying districts of the Republic increase the possibility of infection of Zone employees.

Fifteen species of *Anopheles* mosquitoes are known to be present in the Canal Zone, as compared with the eight species known in 1910. The formation of Gatun Lake has created extensive breeding areas for *A. albimanus*, which will fly at least 20 or 25 miles into sanitated areas. Some of the recently found species have been shown to be very susceptible to infection with the malaria parasites, and of these, *A. punctimacula*, a jungle breeder, may be especially important as a vector.

The incidence of malaria among the military personnel is especially high. The young white soldiers, on temporary duty in the Canal Zone, are more likely to be bitten by infected mosquitoes than civilian employees because of their official and unofficial visits into the Republic, and because of their official duties in unsanitated parts of the Canal Zone, as well as in sanitated army posts, which are situated nearer the unsanitated areas than are the communities of civilian employees.

This book should be of considerable interest to malariologists. The authors have shown clearly how new problems in malaria control are created by changing conditions, and that even in a region such as the Canal Zone, which has become famous for the success of campaigns carried out in it against mosquitoes and mosquito-borne diseases, and where large sums of money have been spent for mosquito control, there can be no let-up in the fight against malaria. The authors wisely plead for the additional information that is necessary before control measures should be directed specifically against the mosquitoes recently suspected as being malaria carriers, as their breeding habits differ from those of the chief vector, *A. albimanus*.

The book is well written, with few printer's errors. The reviewer would like to point out that three of the eleven species of *Anopheles* that the authors state were present in Panama in 1910 are synonomous with other species in the list, so that actually only eight were known at that time. A sixteen page bibliography at the end of the book should be of value to those interested in the history of malaria, and to research workers.

L. E. R.

Health Officers' Manual. By. J. C. Geiger, M.D., Dr. P. H. 148 pp. Illus. $1.50. *W. B. Saunders Company, Philadelphia, 1939.*

This little book is an authoritative outline of current public health practice. Because of the exceedingly brief treatment of various aspects of the subject, however, it is likely to prove of limited value to the novice, while most of the subject matter is already familiar to the experienced health officer. For example, the total space allotted to the discussion of the control of syphilis and other venereal diseases is scarcely more than one page. Perhaps the book's principal value is in providing a bird's-eye view of the relationships of different public health activities to each other.

T. B. T.

Marihuana. America's New Drug Problem. A Sociologic Question with Its Basic Explanation Dependent on Biologic and Medical Principles. By ROBERT P. WALTON. 223 pp. Illus. $3.00. *J. B. Lippincott Company, 1938.*

Here in one volume is collected a great deal of information not only upon the chemistry, pharmacology, and toxicology of hashish but a great deal of interesting information in regard to the history of the drug, the present status of the marihuana vice in the United States, and the methods of using the drug by addicts. The

volume can be read with profit not only by those interested in medicine but also by the criminologists and sociologists.

E. K. M., Jr.

Microbiology and Pathology. By CHARLES F. CARTER. 2nd ed. 755 pp. Illus. $3.25. *C. V. Mosby Company, St. Louis, 1939.*

The first edition of this book was an outgrowth of a book written in 1928, entitled *Bacteriology for Nurses.* The present edition, like the first, is designed to present a rather complete picture of microbiology as a whole and of pathology, so far as it can be taught to student nurses. It is arranged so that it can be used advantageously by schools following the outline of the Committee on Education of the National League of Nursing Education.

The presentation is appealing to the student. The subject matter is clearly and interestingly, though briefly, presented. The many illustrations are good and chosen so as to make use of visual learning processes. Review questions and laboratory exercises given at the end of each chapter should be useful to the student and may be an aid in teaching. References are limited to names of textbooks of more advanced nature. An excellent glossary of terms is appended.

In addition to its usefulness as a text this book should be valuable as a reference for the graduate nurse's personal bookshelf.

R. D. R.

THE PLACENTAL TRANSMISSION OF SULFANILAMIDE AND ITS EFFECTS UPON THE FETUS AND NEWBORN

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Since the introduction of sulfanilamide in the treatment of puerperal sepsis caused by the beta hemolytic streptococcus, the drug has assumed a position of increasing prominence in the armamentarium of the obstetrician. It is also widely used now as a therapeutic agent against various intercurrent infections complicating pregnancy. In an earlier communication, (Speert, 1938), it was reported that sulfanilamide when administered to women in labor passes readily through the placenta, and after three to five hours exists in equal concentrations in fetal and maternal blood. The object of the present investigation was to determine the effects upon the fetus following the administration of sulfanilamide to the mother during pregnancy.

Infants born following a single dose of sulfanilamide to the mother appeared to have suffered no effects attributable to the drug, and their neonatal development was normal in all respects. However, it has been a matter of uncertainty whether prolonged administration of sulfanilamide during pregnancy adversely affects the fetus, and whether the safety of prolonged therapy can be judged solely by the criteria of toxicity usually applied to the adult.

In an effort to obtain data which might throw light upon these questions, rats were fed sulfanilamide throughout pregnancy, and the size of the litters, birth weight, mortality, and rate of growth of the offspring were compared with controls.

METHODS AND MATERIALS

Mature female rats of McCollum's hooded strain, ranging in age from seven to nine months and averaging 200 to 240 grams in weight,

were used. The experimental animals were fed a two per cent mixture of sulfanilamide in the McCollum diet, which consists of whole wheat flour, milk powder, casein, calcium carbonate, and sodium chloride. The controls received this diet without sulfanilamide added. Greens were given at regular intervals. Food and water were always available in excess of the amounts desired by the rats, and they were allowed to partake of same ad libitum. Daily records were kept of weight and of food consumption.

Matings were carried out by placing a male in a cage with several females. Vaginal smears were then made once daily and the presence of sperms taken as the criterion of copulation. As soon as sperms were obtained, the female was placed in a separate cage and started on either a sulfanilamide-free control diet or a sulfanilamide-containing diet, which was not discontinued until parturition, following which all the mothers were fed the sulfanilamide-free diet.

Fifty-three adult rats were employed in this study. Thirty pregnant animals were divided into two equal groups, one of which was maintained on the sulfanilamide diet, the other being used as controls. Seventeen non-pregnant rats were observed for a period of twenty-two days while on the sulfanilamide diet, and were compared with six non-pregnant controls, with respect to food consumption, weight changes, and general behavior during this period.

Determinations of blood sulfanilamide concentrations were made at weekly intervals on 0.02 cc. of blood, obtained from the clipped tail of the adult rat, and analyzed according to the most recent method described by Bratton and Marshall (1939).[1] In four instances sulfanilamide determinations were also made on the heart blood of newborn sacrificed immediately after delivery.

The cages of the pregnant animals were inspected twice daily, at 9:00 A.M. and 6:00 P.M. It was thus possible to ascertain the presence of a newly-born litter soon after delivery. In several instances a newborn was withdrawn from the litter immediately after birth and sacrificed for histologic examination of the tissues.

The newborn in each litter were counted and jointly weighed as

[1] Colorimetric readings were made by Miss Dorothea Babbitt, through the courtesy of Dr. E. K. Marshall, Jr., in a photo-electric colorimeter designed and built by Dr. Morris Rosenfeld (1939).

soon as possible following delivery, and daily thereafter. All the young were weaned at the age of twenty-one days, and were fed the standard McCollum diet until thirty-five days of age, which marked the termination of the period of observation.

The following data are based upon observations on eighty rats born to fifteen mothers who received sulfanilamide during pregnancy, and 125 rats born to fifteen controls.[2]

OBSERVATIONS

Placental transmission

In order to test the placental transmission of sulfanilamide from mother to fetus in the rat, two full-term newborn were taken from each of two litters, and sulfanilamide determinations done on the heart blood. The drug was found to be present in equal concentrations in the blood of the mother and of the newly-born littermates. Thus in one animal, the maternal blood sulfanilamide level on the day of delivery was 36.0 mgm. per cent, and the concentrations in the blood of the two newborn were 36.2 mgm. per cent and 36.7 mgm. per cent. In another animal the maternal blood sulfanilamide concentration five days before delivery was 24.0 mgm. per cent. At birth the blood levels of the two offspring were 27.2 mgm. per cent and 28.0 mgm. per cent, respectively.

Fetal deaths

Death of the litter in utero occurred in at least three of the fifteen sulfanilamide-fed pregnant rats. In two cases macerated or resorbing fetuses were found in the uteri of mothers sacrificed at term. In another animal parturition did not occur until the twenty-fifth day of pregnancy (three days past term), when a partially eaten, term sized, macerated newborn was found in the cage. In still another

[2] It is a common procedure for the mother rat to eat any dead young present in the cage. Since in many cases several hours elapsed between parturition and inspection of the cages, it was impossible to gain an accurate determination of the total number which comprised each litter at birth, if the stillborn and early neonatal dead had been eaten. It seemed convenient therefore, for purposes of comparison, to consider only the live newborn found in the cages at the first examination.

case pregnancy was believed to have terminated prematurely at nineteen days, at which time fresh bleeding from the vagina was noted, together with a sudden decline in maternal body weight. Since no young were found in the cage, it was assumed that they had been eaten by the mother. These abnormalities were not observed among the fifteen control pregnancies.

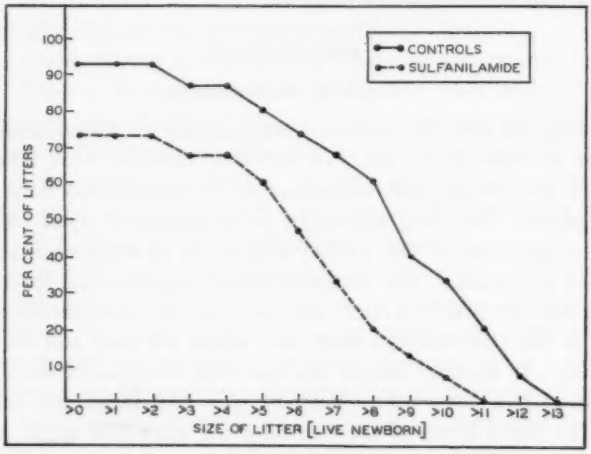

FIG. 1. DISTRIBUTION OF SIZES OF LIVE LITTERS BORN TO SULFANILAMIDE-FED MOTHERS, AS COMPARED WITH CONTROL LITTERS

Size of litter

When the experimental animals were compared with the controls, a significant difference between the average sizes of the litters was noted. The sulfanilamide-fed rats averaged 5.3 live newborn per litter, whereas in the control series, the average litter size was 8.3. In the experimental series, twelve litters (80 per cent) consisted of eight or less live newborn per litter. Among the control animals, on the other hand, ten litters (67 per cent) contained from eight to thirteen live young per litter. The distribution of the litter sizes is shown graphically in figure 1.

Birth weight

The average weight per newborn revealed a striking difference between the sulfanilamide series and the controls, the latter weighing

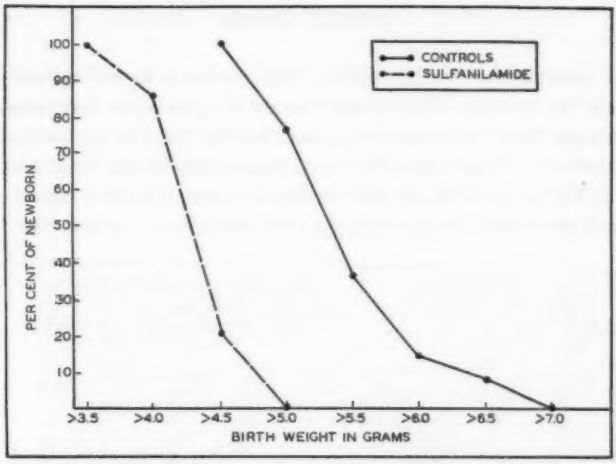

FIG. 2. DISTRIBUTION OF BIRTH WEIGHTS AMONG THE YOUNG BORN TO SULFANILAMIDE-FED MOTHERS, AS COMPARED WITH CONTROLS

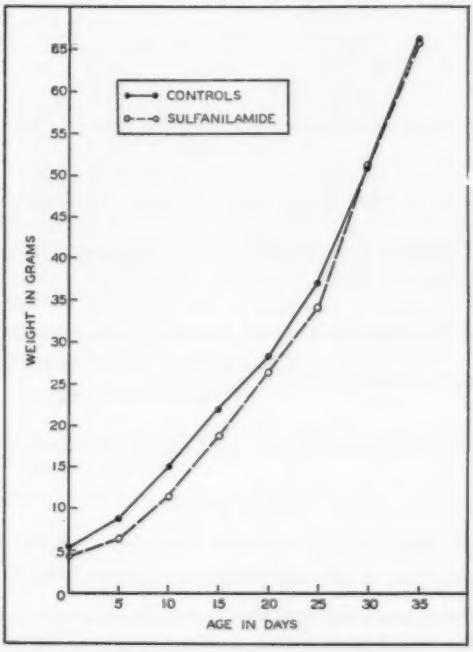

FIG. 3. POSTNATAL WEIGHT CURVES BASED ON SURVIVING RATS

27 per cent more. Of the eighty live rats born to sulfanilamide-fed mothers the average birth weight was 4.35 grams, as compared with an average birth weight of 5.52 grams for the 125 live newborn of control mothers. That this difference was a consistent finding is indicated in Figure 2, which shows the distribution of birth weights. One hundred per cent of the sulfanilamide newborn weighed five grams

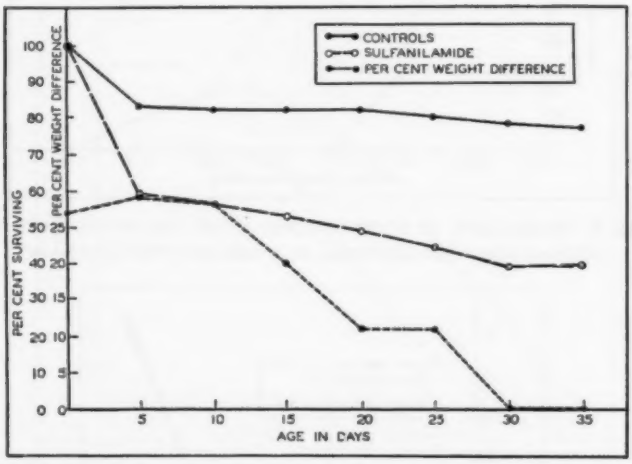

FIG. 4. SHOWING (A) THE PERCENTAGE INCIDENCE OF SURVIVAL AMONG THE SULFANILAMIDE YOUNG AS CONTRASTED WITH THAT IN THE CONTROL SERIES; AND (B) THE WEIGHT DIFFERENCES IN THE TWO GROUPS DURING THE FIRST THIRTY-FIVE DAYS OF LIFE

The weight difference is expressed as the percentage by which the control young exceeded the sulfanilamide group, according to the formula:

$$\text{per cent weight difference} = \frac{\text{control weight} - \text{experimental weight}}{\text{experimental weight}} \times 100$$

or less at birth, whereas 77 per cent of the controls exceeded this weight.[3]

Rate of growth

The growth rate of the newborn revealed a definite lag in the average weight gain of the experimental animals behind the controls.

[3] Since each litter was weighed as a unit, the average weight of the individuals comprising it determined the allocation of the entire litter in the weight groupings.

At birth the latter weighed 27 per cent more than the sulfanilamide animals, and this advantage was maintained throughout the first ten days of life (fig. 4). By the fifteenth day the difference had been reduced to 20 per cent; and from this point on, a gradual approximation of the growth curves occurred (fig. 3), so that by the thirtieth day the average weights of the surviving animals in both groups

FIG. 5. LITTERMATES, 10 DAYS OLD, BORN TO MOTHERS WHO RECEIVED SULFANILAMIDE DURING PREGNANCY, SHOWING INDIVIDUAL VARIATIONS IN GROWTH OF THE YOUNG

were identical, and remained so until the thirty-fifth day, when the observations were terminated.

The controls exhibited only slight variations in weight gain among littermates, and the increase in size was fairly uniform for all the individuals of a given litter. Among the sulfanilamide young, however, striking variations in growth among individuals of the same litter frequently occurred. In many of the experimental litters several of the rats exhibited markedly stunted growth and retarda-

tion in weight gain, while their littermates showed relatively normal development (fig. 5). In some of the experimental litters all were similarly stunted. This stunting effect was found to occur to varying degrees (figs. 7 and 8), and in some cases was so striking that the runts could be singled out at birth or within the first few days of life

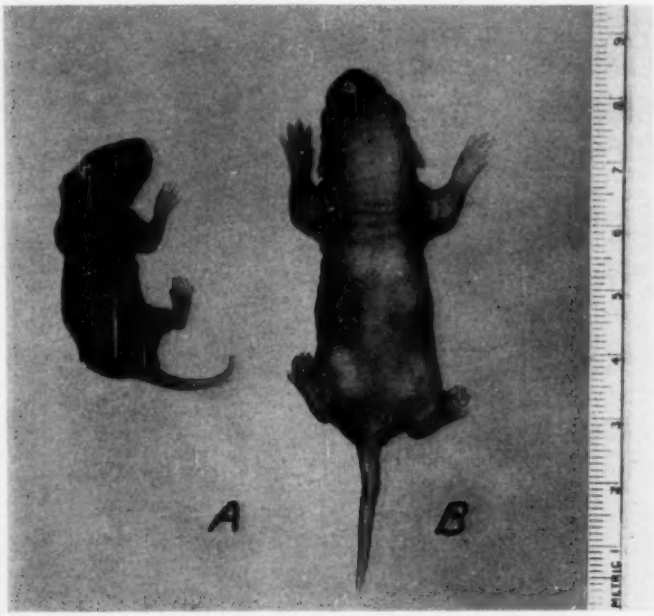

FIG. 6. FOUR-DAY-OLD RATS, SHOWING STUNTING EFFECT OF SULFANILAMIDE UPON GROWTH OF EXPERIMENTAL NEWBORN

A, born to sulfanilamide-fed mother, *B*, normal control

(fig. 6). With increasing age, the contrast between the runts and the normal controls of the same age usually became more marked (figs. 6 and 7).

Mortality

The mortality rate among the sulfanilamide newborn was strikingly higher than that of the controls. During the first five days

of life the mortality (corrected for newborn sacrificed at birth) was 17 per cent for the controls. In marked contrast to this was a 41 per cent mortality for the sulfanilamide newborn. Thus, at five days only 59 per cent of the live-born experimental animals remained alive,

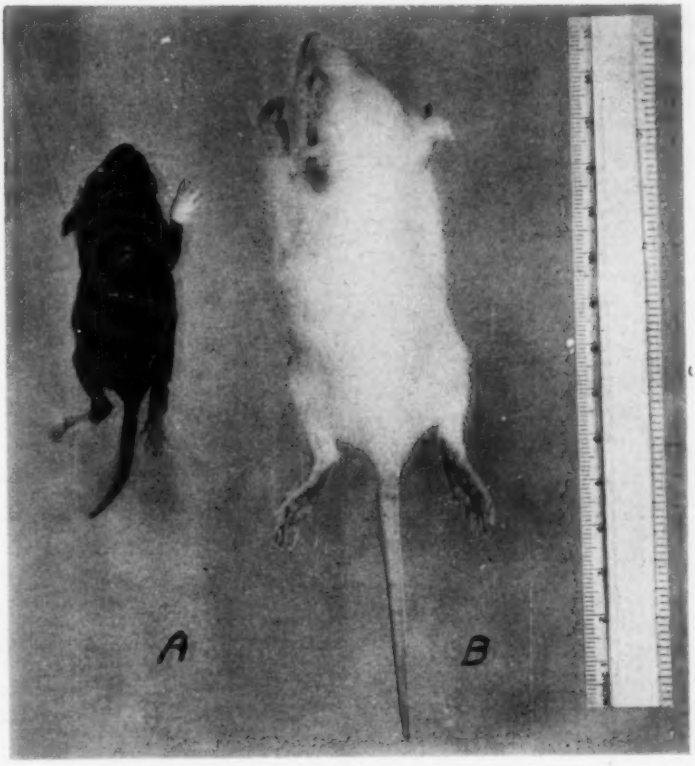

FIG. 7. SAME ANIMALS AS IN FIG. 6. AGE 21 DAYS

whereas 83 per cent of the live-born controls survived this period. Following this precipitous drop, the survival curve for the sulfanilamide series exhibits a gradual decline, while the control survival curve remains practically flat (fig. 4). Thus, at thirty-five days 77 per cent of the controls remained alive, while in striking contrast is

a survival incidence among the sulfanilamide animals of only 39 per cent. The mortality was particularly high among the stunted individuals in the sulfanilamide group.

FIG. 8. SEVENTEEN-DAY-OLD RATS, SHOWING STUNTING EFFECT, BUT TO A LESSER DEGREE THAN IN FIGS. 6 AND 7
A, normal control, *B*, born to sulfanilamide-fed mother

A somewhat different approach to an analysis of the comparative mortality rates of the control and experimental groups of animals is through a comparison of the average sizes of the surviving litters. At birth the sulfanilamide litters averaged 5.3, as compared with 8.3 for the controls. This relative difference became progressively wider

(table 1), due to the higher mortality among the sulfanilamide young. By twenty-five days the average control litter was three times as large as the average experimental litter. At thirty-five days the sulfanilamide litters averaged only 2.1 animals (corrected for those sacrificed at birth), in contrast to an average of 6.4 (corrected) in the control series.

No correlation could be established between maternal blood sulfanilamide concentrations during pregnancy, on the one hand, and birth weight, size of litter, degree of stunting of the young, or mortality, on the other.

TABLE 1

Average size of surviving litters at successive periods of postnatal life

AGE	SULFANILAMIDE	CONTROLS
days		
Birth (live)	5.3	8.3
5	2.9 (3.1)*	6.7 (6.9)
10	2.8 (3.0)	6.6 (6.8)
15	2.6 (2.8)	6.6 (6.8)
20	2.4 (2.6)	6.6 (6.8)
25	2.1 (2.3)	6.5 (6.7)
30	1.9 (2.1)	6.3 (6.5)
35	1.9 (2.1)	6.2 (6.4)

* Figures in parentheses denote values corrected for the young sacrificed at birth.

Examination of the tissues of the newborn sacrificed at birth revealed no significant gross or microscopic differences between the controls and the experimental animals.

The average daily food consumption among the pregnant animals did not differ significantly between the control and the sulfanilamide-fed groups.

The daily sulfanilamide intake averaged approximately one gram per kilogram of body weight. This is a much higher dosage than is recommended for therapeutic use in the human, but due to poorer absorption from the gastro-intestinal tract of the rat (Marshall and Cutting, 1938), it results in a relatively lower blood level. Fifty determinations of blood sulfanilamide concentrations showed a nor-

mal distribution, with a mean of 19.7 milligrams per cent and a standard deviation of ±8.1.

Seventeen non-pregnant rats on the sulfanilamide diet lost an average of 16 grams per animal during the twenty-two days of observation. Six non-pregnant controls gained an average of 4 grams each during this same period. Among the pregnant animals the average weight gain during the period of gestation (twenty-two days) was 32 grams for the sulfanilamide series and 72 grams for the controls. The behavior of the animals receiving sulfanilamide differed in no respect from that of the controls; all the animals appeared entirely normal during the period of observation. Histologic sections of various portions of the gastro-intestinal tract revealed normal mucosae. The spleens of the sulfanilamide-fed animals were slightly enlarged, however, darker than normal, and contained increased quantities of hemosiderin on microscopic examination.

DISCUSSION

The use of sulfanilamide in the treatment of intercurrent infections during pregnancy has heretofore been considered largely from the viewpoint of the maternal indications and contra-indications. The intrauterine effects of the drug upon the fetus have remained a matter of conjecture, and in most cases it has been tacitly assumed that no specific toxic effect is exerted. In an attempt to put this assumption to an experimental test, pregnant rats were fed sulfanilamide throughout the twenty-two day period of gestation, and the offspring were compared with controls until the thirty-fifth postnatal day. The resultant observations clearly indicate that the chronic administration of the drug to the mother during pregnancy produces deleterious effects upon the rat fetus.

The average birth weight in each litter of rats born to sulfanilamide-fed mothers was consistently less than that of the controls. This difference was largely due to the presence of numerous runts among the former. Maternal malnutrition during pregnancy suggested itself as a possible factor responsible for the dwarfing of the experimental animals. Yet the food consumption of the sulfanilamide-fed mothers was equal to that of the controls, and histologic sections of the gastro-intestinal tract revealed no anatomical changes which

might suggest defective absorption. The experimental mothers all gained weight during pregnancy, although distinctly less than the controls, which was due principally to the smaller size and weight of the experimental litters. It seems clear, therefore, that maternal malnutrition played no rôle in the production of the dwarfed rats born to the sulfanilamide-fed mothers.

The average rate of growth of the experimental young showed a significant lag behind that of the controls. This also was due to the presence of the runts in the sulfanilamide litters. As the stunted animals began to die off, the growth curve of the experimental animals began to approach the control growth curve, and at the age of thirty days, by which time practically all of the dwarfed sulfanilamide rats had succumbed, the average weight of their surviving, normally developing littermates equalled that of the controls. From this point on, the experimental and control growth curves coincided.

In an attempt to ascertain the cause of the stunted offspring of the sulfanilamide-fed mothers, the possibility of impaired maternal lactation was considered. However, in many instances it was possible to detect the runts at birth, before there had been any opportunity for significant weight gain due to suckling. Moreover, if defective lactation were responsible for the stunted growth, one would expect a uniform effect upon all the individuals of a given litter. Within the first few days of life, however, it became easy to differentiate all the runts from their normally growing littermates. It seems clear therefore, that one must look elsewhere than to maternal mammary function for an adequate explanation for the failure of many of the experimental young to develop at a normal rate of growth.

Further evidence of the noxious effect of sulfanilamide upon the fetus is found in the high mortality among the experimental young. Death in utero of the entire litter occurred in one-fourth of the sulfanilamide-fed animals, in most cases before the stage of viability had been reached. The average litter size of the live-born controls was 57 per cent greater than that of the sulfanilamide series. This fact serves as further indication of the existence of conditions suboptimal for intra-uterine development.

The postnatal mortality rates revealed an equally striking difference between the experimental and control groups of rats. During

the first five days of life 41 per cent of the sulfanilamide series succumbed, as compared with only 17 per cent of the control group. This observation is in accord with the findings of Adair, *et al.* (1938), who noted an increased neonatal mortality rate among the young born to rabbits who had received sulfanilamide during the latter part of pregnancy. However, their results are complicated by a high maternal mortality, as well as by the fact that the mothers lost large amounts of weight during the period of sulfanilamide administration. It appears also, from the brief account given, that faulty maternal lactation may have been an important factor responsible for their high neonatal mortality.

It is interesting to note that in the present experiments the per cent weight difference between the control and experimental groups of young showed no significant changes during the first five days of life. This suggests that the deaths in the sulfanilamide group were proportionately distributed among the runts and their normal appearing littermates, rather than confined to the former group. It might therefore be inferred, that during the first few days of life a general toxic effect, independent of the selective stunting of growth, was exerted upon the experimental young.

Following the sharp decline in the sulfanilamide survival curve, illustrative of the high mortality among the experimental offspring during the first few days, there occurs a gradual, steady taper until the age of thirty days; during this period the survival curve of the controls remains practically flat. Thus, at thirty days the incidence of survival among the experimental young was only one-half, and the average litter size only one-third, that of the controls. This "secondary" mortality occurred almost exclusively among the sulfanilamide runts. The average weight of the surviving experimental offspring therefore approached more closely that of the controls, and the per cent weight difference between the two groups became progressively smaller.

It has been amply demonstrated that sulfanilamide, when given to pregnant women at term, passes readily through the placenta and reaches equilibrium between fetal and maternal blood (Barker, 1938; Speert, 1938; Digonnet, 1939). In the present experiments it was found that the rat placenta at term is likewise freely permeable to the

drug. Lee, *et al.* (1938) demonstrated the placental transmission of sulfanilamide in rabbits twenty-three days pregnant (nine days before term), and in unpublished experiments the writer has found the drug to pass through the rabbit placenta at various earlier stages of pregnancy with a rapidity equal to that at term. No data are available concerning the placental transmission of sulfanilamide in the human during earlier stages of pregnancy. However, sulfapyridine, a compound closely related chemically to sulfanilamide, was found in approximately equal concentrations in fetal and maternal blood, following a five months spontaneous abortion in a patient who had been receiving the drug for ten hours. Since the human, rat, and rabbit placentas are all of the hemochorial type and possess many structural similarities (Grosser, 1927), it is reasonable to assume that the placental transmission of sulfanilamide occurs also in the rat at early stages of pregnancy. This consideration, which is obviously of fundamental importance in the interpretation of the present experimental findings, becomes of further interest in the light of recent experiments which showed the placental transmission of another chemotherapeutic agent, neoarsphenamine, to vary with the stage of pregnancy (Snyder and Speert, 1938).

The dosage of sulfanilamide employed in the present study was without apparent ill effect on the adult animals. This agrees with previous reports where rats were fed comparable amounts of sulfanilamide over prolonged periods with no impairment of appetite or other signs of toxicity (Rimington and Hemmings, 1938; Molitor and Robinson, 1939). The minor weight loss in our non-pregnant sulfanilamide-fed animals may be explicable on the basis of a diuresis, observed by Rimington and Hemmings in rats receiving the drug. The increased deposition of hemosiderin in the spleen in sulfanilamide-fed animals has been observed by many investigators, but its exact significance is not yet clear.

In summary, when sulfanilamide is administered to pregnant rats in amounts well within the limits of toxicity, markedly injurious effects are produced in the fetuses, demonstrated by increased intrauterine and postnatal mortality, decreased litter size, decreased birth weight, and striking, selective stunting among the offspring. Since sulfanilamide was found to pass freely from the maternal to the fetal

bloodstreams, it seems reasonable to attribute these effects to the presence of the drug in the fetal circulation. Although acetylation of sulfanilamide occurs to only a slight extent in the rat, and is therefore not likely to alter significantly conclusions based on blood concentrations of the free drug (Marshall and Cutting, 1938), it should be borne in mind that acetyl-sulfanilamide is a more toxic substance and, as such, may be partly responsible for the effects produced on the fetus.

Since the stunting effect manifested itself as a selective process, involving some members of a litter and not others, and since sulfanilamide was found in equal concentrations in the blood of littermates, it seems likely that individual variations in susceptibility to the drug occur among the fetuses.

It is hazardous to carry over to one species conclusions from experimental observations made upon another. Moreover, in the present experiments the blood sulfanilamide levels were much higher than usually reached in the treatment of human patients. Also the administration of the drug was continued throughout gestation, in contrast to the relatively shorter periods of therapy that are usually required clinically. No claims can therefore be made for any clinical application of these experimental findings. Until more data are available concerning the effects of sulfanilamide upon the human fetus, however, animal experimentation must be resorted to for predictions of human fetal toxicity. Prolonged or indiscriminate use of sulfanilamide during pregnancy is therefore cautioned against, on the basis of the present demonstration of its undesirable effects on the fetal and newborn rat.

CONCLUSIONS

1. The administration of sulfanilamide to rats throughout gestation causes it to appear in equal concentrations in the blood streams of the mother and the fetuses.

2. Prolonged administration of sulfanilamide to pregnant rats produces deleterious effects upon the offspring. These effects include increased intra-uterine and postnatal mortality, decreased litter size, diminished birth weight, and selective stunting of growth.

3. Until further observations have been made of the effects of

sulfanilamide upon the human fetus, the drug should be administered with extreme caution during pregnancy. Such observations should include a careful study of intra-uterine development, birth weight, and postnatal growth in the infants born to mothers receiving extended sulfanilamide therapy during gestation.

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SEVERE AORTIC INSUFFICIENCY IN ASSOCIATION WITH A CONGENITAL MALFORMATION OF THE HEART OF THE EISENMENGER TYPE

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Congenital aortic insufficiency is extremely rare. There are, however, two types of congenital malformations of the heart in which it is usual to find signs of aortic insufficiency. The first (1, 3) is when coarctation of the aorta occurs in conjunction with bicuspid aortic valves and sub-aortic stenosis; this combination of anomalies is commonly accompanied by signs of aortic insufficiency. In these cases the aortic insufficiency is generally considered not to be congenital in origin, but due to a healed bacterial endocarditis. The other type (1) of congenital malformation which is accompanied by signs of an aortic insufficiency is when a congenital aneurysm of the wall of the right sinus of Valsalva ruptures into the right ventricle; such a perforation enables blood to flow from the aorta into the right ventricle during diastole. The following case is reported because the aortic insufficiency was associated with a congenital malformation of the heart of the Eisenmenger type. In this case the aortic cusps were found to be abnormal in size and position. The line of closure of the right aortic cusp was retracted into a septal defect, thus rendering the aortic valve grossly insufficient.

The occurrence of the aortic insufficiency in the absence of either of the two types of congenital malformations mentioned above, always necessitates the consideration of an acquired infection. The most common cause of this condition in childhood is acute rheumatic fever. The possibility of syphilis must also be considered. Although acquired syphilis is a frequent cause of aortic insufficiency, the authors know of no cases in which aortic insufficiency has been proved to be due to congenital syphilis.

In the adult the possibility of rupture of the aortic valves due to

trauma or strain, although rare, deserves consideration in cases of insufficiency of an unusual and extreme nature. Such accidents, however, have never been known to occur in childhood.

The other well recognized cause of aortic insufficiency is a non-rheumatic bacterial infection, either acute or subacute. Indeed, in the present case, an acute ulcerative endocarditis was thought to be the cause of the aortic insufficiency. At autopsy, however, it was evident that the deformed aortic valve was not involved in the acute terminal endocarditis. Because of its unusual nature the case is reported in detail.

CASE REPORT

F. L. (H. L. H. No. A-6409) was a 7-year-old negro boy who was brought to the Harriet Lane Home on March 19, 1938 with the complaint of headache and high fever.

Family history was non-contributory. There was no history of syphilis nor of acute rheumatic fever.

Past history: The birth was normal; weight 7½ pounds. No cyanosis, no eruptions, no snuffles, no hemorrhages were noted in the neonatal period. The feedings were adequate. The development was normal. The child had had pertussis at 3 years and measles and chickenpox at 4 years. There was no rheumatic history nor had the boy ever suffered from severe tonsillitis or growing pains.

Three weeks prior to admission, as a result of a routine school examination, a "heart examination" was advised. The boy had always appeared healthy and had never complained of palpitation nor of dyspnea. He had led a normal active life.

Present illness dated from five days before admission when the patient developed headache and high fever. The following morning he seemed better but toward evening became weak and drowsy; both that night and the following night the patient had a high fever. The day before admission the mother thought the child's heart was beating rapidly. The following day she took the child to her doctor who immediately referred him to the hospital.

Physical examination: Temperature 40°, pulse rate 120, respirations 36, blood pressure 112/0.

The *general appearance* was that of a moderately well nourished, well developed colored boy who looked acutely but not critically ill. The skin was hot and dry. There was no rash, no petechiae and no visible cyanosis. The pulse was of an extraordinarily collapsing quality; the pulse in the dorsalis pedis could be readily palpated through the bed-clothes. The head shook with each cardiac impulse. The eyes, ears and nose were normal. The teeth were carious; the tonsils small and not acutely inflamed. The pulsations in the carotid arteries were conspicuous and the episternal notch bulged with each systole.

Examination of the *chest* showed a tremendous precordial heave. The *heart* was enormously enlarged both downward and outward. Over the precordium there was a pronounced systolic thrill which was maximum at the base along the right border of the sternum. In addition there was a pronounced diastolic thrill palpable at the base; this, too, was more easily palpated to the right than to the left of the sternum. The systolic thrill was transmitted not only to the vessels of the neck, but it was even palpable in the femoral arteries.

On auscultation there was a very loud, ringing aortic diastolic murmur heard over the sternum, maximum on the right side. The second sound was completely obliterated. In addition there was a very harsh systolic murmur heard all over the precordium, maximum over the base of the heart close to the sternum. It was this systolic murmur which was transmitted to the vessels of the neck and the femoral artery. The sounds at the apex were remarkably quiet. The rate was irregular due to numerous extrasystoles.

The *lungs* were resonant. The breath sounds were so weak that they were inaudible above the heart sounds.

The *liver* was two fingerbreadths below the costal margin. The tip of the *spleen* was palpable. The *extremities* showed a pronounced capillary pulse but no clubbing, no cyanosis and no rheumatic nodules.

Laboratory examination showed the following significant findings: Red blood count was 3,850,000; white blood count was 7,250 with 56 per cent polymorphonuclear cells and 24 per cent lymphocytes. The urine showed a trace of albumin; specific gravity was 1.018. There was no sugar or acetone; the microscopic examination of the sediment showed no red blood cells, no white blood cells and no casts. The Wassermann reaction of the blood was negative. The corrected sedimentation rate was 25 mm. The teleoroentgenogram showed the heart to be enlarged and "boot shaped" (Plate 1). The cardiac shadow extended 9.3 cm. to the left and 4.4 cm. to the right of the mid-sternal line; the total transverse diameter of the chest was 19 cm. The electrocardiogram showed a rate of 125; a normal sinus mechanism; a P-R interval of 0.16–0.18 of a second; notching and slight prolongation of the QRS interval in lead 1 (0.06–0.08). The blood culture showed a heavy growth of staphylococcus aureus; a culture from a nasal swab also showed staphylococcus aureus.

Clinical impression: Congenital malformation of the heart and a staphylococcus septicemia. It was thought probable that the superimposed bacterial infection had caused an ulcerative endocarditis and an aortic insufficiency. Cardiac failure due to a staphylococcus myocarditis. Three possible malformations were considered to account for the extensive involvement of the aortic valves, namely,—(1) subaortic stenosis, (2) bicuspid aortic valves and (3) congenital aneurysmal dilatation of the sinus of Valsalva.

Course in the hospital: The temperature was high and irregular, varying between 39° and 41°. The heart sounds became more confused and a friction rub developed at the apex. The liver showed progressive enlargement and became extremely

tender. The urine showed an increasing amount of albumin and a few casts. During the last few hours of life the patient developed signs of consolidation over the left lower lung. He died five days after admission.

Clinical impression at time of death: Staphylococcus septicemia; congenital malformation of the heart; ulcerative endocarditis of aortic valve; staphylococcus myocarditis; pulmonary infarcts and metastatic abscesses to the liver.

Autopsy no. 15826. The major interest centered about the *heart* which occupied most of the left thoracic cavity. The apex pressed against the left chest wall. The unopened pericardial sac measured 16 cm. in its greatest diameter. It contained about 100 cc. of straw-colored fluid. The great vessels appeared to occupy their normal position. The external surface of the heart was smooth. The coronary arteries were patent throughout their course. The ductus arteriosus was completely obliterated. The auricles were of normal size; the ventricles, especially the left, were greatly enlarged.

When the heart was opened, immediately beneath the right aortic cusp there was a gross defect in the interventricular septum. The aorta partially overlay the septal defect, thus establishing direct continuity with the right ventricle. The right sinus of Valsalva, i.e., that which corresponded in position with the right coronary orifice, was greatly increased in size; its deepest portion measured 2 cm. and its cusp measured 3.5 cm. along the line of closure. The neighboring left sinus of Valsalva was also abnormally large. It measured 1.5 cm. in depth and its cusp measured 2.5 cm.; whereas the third sinus measured but 1.0 cm. in depth and its cusp 2.0 cm. The margin of the right aortic cusp was thickened and firm; its line of closure was markedly retracted and lay at a lower level than that of either of the other two cusps. In addition, at both insertions this cusp was densely adherent to the adjacent cusps, thereby making the aortic valve grossly insufficient (Plate 2, fig. 1). Microscopic sections of these showed a thickened valve at the line of closure with slight hyalinization and infiltration with connective tissue cells. The aortic ring measured 6.0 cm. The coronary orifices seemed normal but stood rather high in the sinuses. The aorta distal to the valves was of normal appearance. The aortic orifice, as previously mentioned, was displaced so that it partially overlay the right ventricle.

This abnormal position of the aortic orifice rendered it impossible for the aortic septum to meet the ventricular septum. Consequently there was a defect at the base of the ventricular septum. The defect measured 1.75 cm. in diameter. Along the lower margin of the defect the endocardium was thickened and rolled and showed minute vegetations. Microscopic examination of a section taken from this area showed an endocarditis with necrotic leucocytes and many gram positive bacteria. Beneath this area there was round cell infiltration and necrosis of the myocardium. The necrosis, however, was superficial and limited to this

small area. The remainder of the myocardium was normal. The left ventricle, although greatly dilated, was essentially normal. The myocardium of the left ventricle was thickened and flecked with yellow beneath the endocardium. The left ventricular wall measured 1.3 cm. in thickness; whereas the thickness of the right ventricular wall was 0.5 cm.

Both auricles were normal; the foramen ovale was completely closed. The ventricular surface of the aortic leaflet of the mitral valve was roughened but the line of closure was delicate; its ring measured 8.5 cm. in circumference. The tricuspid valve was normal except for a hemorrhage at its base; its ring measured 9.0 cm. The chordae tendineae were anomalously placed. Those from the middle segment of the tricuspid valve extended down to a thickened ridge, which in turn extended from a papillary muscle toward the anterior surface of the right ventricle in such a way as to meet another chorda tendinea of the tricuspid valve. The chordae tendineae and the muscular ridge, which occupied the position of the moderator band, tended to separate the pulmonary conus from the main part of the right ventricle at the base of which lay the ventricular septal defect. Thus it was possible for a small amount of blood from the right ventricle to be directed through the septal defect into the aorta. Between the posterior wall of the left ventricle and the base of the right aortic valve, i.e., extending across the interventricular septal defect, there was a curious network of chordae tendineae (Plate 2, fig. 2). The pulmonary conus occupied its normal position and its wall was thin. The pulmonary valve was normal in size and structure; the pulmonary ring measured 6.5 cm. in circumference.

The *left lung* was compressed. The upper lobe was air-containing. The lower lobe showed dark red patches which although not definitely consolidated seemed airless. In addition, there were many scattered hemorrhagic patches throughout both lungs. Microscopic examination showed that the alveoli of both lower lobes were filled with red blood cells. There was an infected thrombus in one of the small branches of the pulmonary veins.

The *liver* contained multiple scattered whitish areas of pin point size. Microscopic examination showed small areas of necrosis which had a tendency to be located about the central veins.

The *spleen* was not enlarged but on microscopic examination it also showed many small areas of necrosis.

The *kidneys* were normal; the kidney pelves were not dilated. Both *ureters*, however, were dilated. The left ureter in its lower two-thirds measured 1.5 cm. in internal circumference. The right ureter measured 2.3 cm. at various levels throughout its length. The *bladder wall* was definitely hypertrophied; it measured 1.0 cm. in thickness. Below the ureteral orifice there was a transverse fold which was slightly higher than normal.

The *other organs* showed no significant gross or microscopic changes.

The *final anatomical diagnosis:* Congenital malformation of the heart. High interventricular septal defect. Slight dextroposition

of the aorta. Abnormally large right aortic cusp and right sinus of Valsalva. Retraction of adjacent aortic cusp. Cardiac hypertrophy and dilatation. Septicemia (staphylococcus aureus). Acute bacterial endocarditis of the septal defect and mitral valve. Necrotic areas in the liver and spleen; infected thrombus in the lung with hemorrhage into the alveoli. Congenital urinary obstruction of the vesicle orifice. Hypertrophy of bladder wall. Bilateral hydroureters.

SUMMARY

The case is one of a negro boy of 7 years of age in whom there were signs of congenital malformation of the heart as evidenced by a rough precordial systolic thrill and murmur maximal at the base which was transmitted not only to the vessels of the neck but also to the femoral arteries. In addition to this, there were signs of extreme aortic insufficiency. The head shook with each cardiac impulse and the pulsations in the dorsalis pedis were readily felt through the bedclothes. The patient was first seen five days before death with a high fever and signs of an overwhelming septicemia. Blood culture revealed a heavy growth of staphylococcus aureus.

At autopsy the boy was found to have a congenital malformation of the heart of the type described by Eisenmenger (2), i.e., a slightly dextroposed aorta which overlay a high ventricular septal defect and a pulmonary artery of normal size. In addition, the aortic cusps, especially that which over-rode the septal defect, were thickened and retracted along their margins, and the line of closure of one cusp lay at a different level from that of the other two cusps. The aortic valve was grossly insufficient. There was evidence of recent inflammation of the ventricular surface of the mitral valve and of the endocardium along the edge of the septal defect; there was, however, no evidence of this acute inflammatory process on the aortic valves. The microscopic hyalinization and fibrosis of the aortic valve indicated that the changes in the valves were old and had long antedated the terminal staphylococcus infection.

Whether the aortic insufficiency dated from birth is not known. At autopsy there was no gross or microscopical evidence of syphilis. The possibility of a healed bacterial endocarditis cannot be excluded. Nevertheless, it seems probable that the aortic insufficiency was in

some way related to the congenital malformation of the heart which involved the base of the aorta. In short, we believe that this case represents another type of congenital malformation of the heart which is liable to be associated with aortic insufficiency.

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PLATE 1

TELEOROENTGENOGRAM AND ELECTROCARDIOGRAM OF PATIENT, F. L.

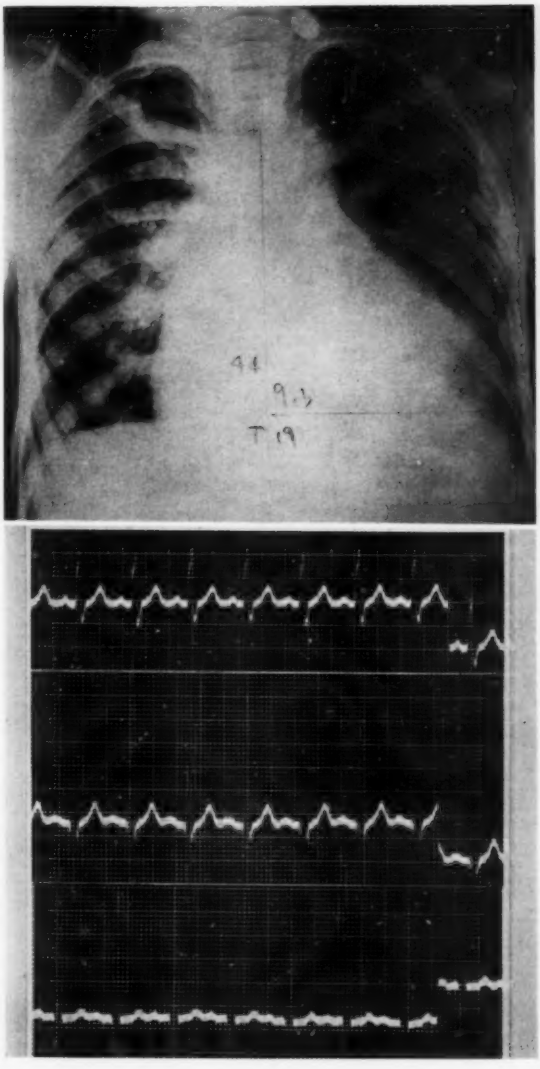
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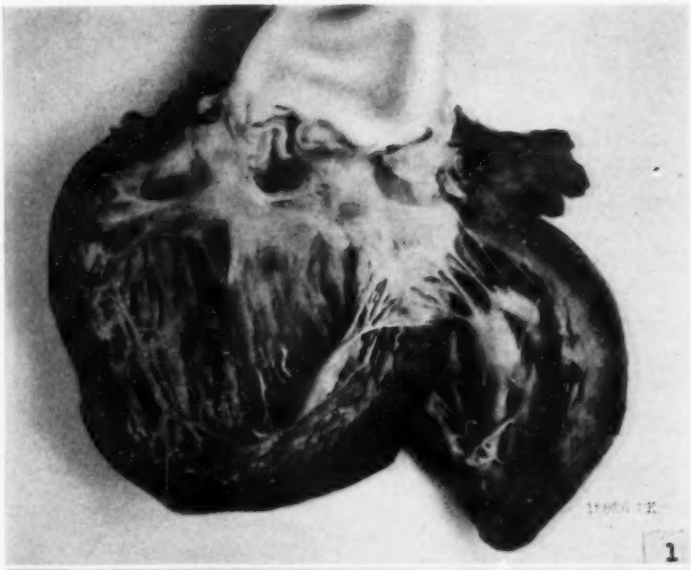
PLATE 2

Photographs of Heart

Fig. 1 shows the interior of the left ventricle and the aortic orifice above the interventricular septum. It also shows the thickening, retraction and the abnormal line of closure of the right aortic cusp immediately above the defect.

Fig. 2 shows the interior of the right ventricle illustrating the manner in which the chordae tendineae stretch across the septal defect.



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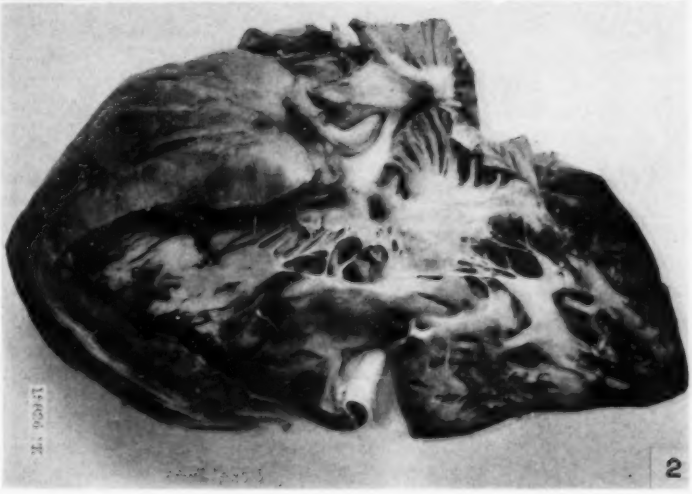

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UNIV. OF MICH.

THE METABOLISM OF TISSUE CULTURES OF WALKER RAT SARCOMA 319[1]

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Cells grown in tissue culture have certain characteristics permitting metabolic study which tissue slices and perfused organs do not possess. In living cultures, as shown in figure 1, the cells are spread out usually in a single layer over a broad surface which condition enables adequate diffusion of materials into and out of the cells. They adjust themselves to conditions for survival and for growth outside the body and are cultured in pure strains. Many types have been isolated and some have been maintained in continuous cultures for many years in our tissue culture laboratory. The strain with which this report deals had been under continuous cultivation *in vitro* for over five years at the time when the experiments to be reported here were done.

Exact knowledge of the metabolic needs for the growth of cells may be obtained by the use of tissue cultures, knowledge which may not be gained by any other method. The method has also disadvantages, the most important of which are the difficulties in growing adequate amounts of tissue for metabolic study and in keeping strict aseptic conditions throughout the growth period. Through the use of the roller tube method, however, (Gey and Gey (8)) some of the difficulties have been overcome and amounts of tissue may be obtained which compare favorably with the weights of tissue used in tissue slice techniques. The methods which have been devised for slices, therefore, may be used with slight modifications in the study of tissue cultures. With the use of the roller tube method for growth, an investigation was made of the oxygen consumption, glucose utilization, lactate production, and urea and non-protein nitrogen

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metabolism of a pure strain of malignant cells, those of the Walker Rat Sarcoma 319.

There were several reasons for selecting this particular strain of cells. Its growth rate is fairly rapid in culture and in susceptible rats which facts make possible a comparison of the results obtained from cells grown in culture with those derived from cells grown in

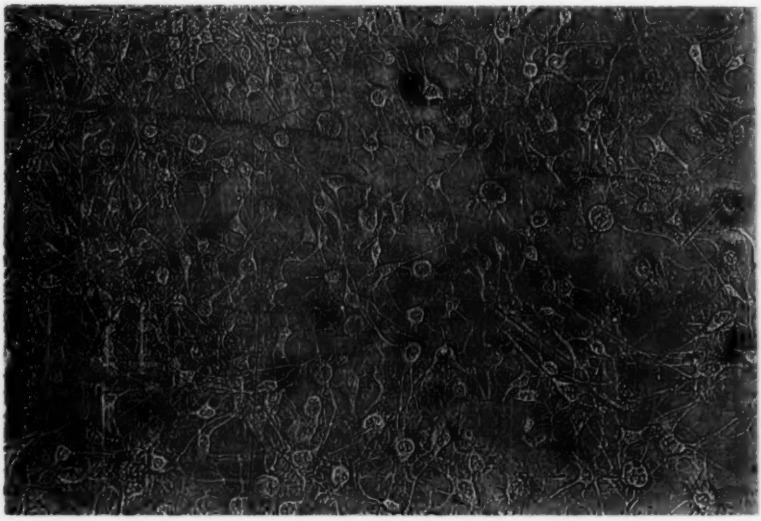

FIG. 1. PHOTOGRAPH OF LIVING CELLS OF WALKER RAT SARCOMA 319 IN PURE STRAIN IN ROLLER TUBE

The multipolar cells of the peripheral zone of the colony are arranged in a single layer. One dividing cell is present in the right hand portion of the field. Mag. ×150.

the animal host. In addition, the observations of other workers on this particular transplantable and explantable tumor may be used for comparison with our observations. When maintained in continuous cultures *in vitro*, the cultural characteristics of the tumor are quite constant. Its cells in pure strains are easily recognized microscopically and are different from those of a number of other races of normal and malignant rat fibroblasts which we have maintained in pure strains under identical conditions.

In early living colonies (figs. 1 and 2) and also in colonies ten days old, the apparently crowded cells in the central area are quite active and healthy; and dividing cells can be found throughout the entire colony. The cells of this tumor grow well in media varying greatly in composition and can be maintained indefinitely in heterologous media with or without a plasma coagulum. Little can be said re-

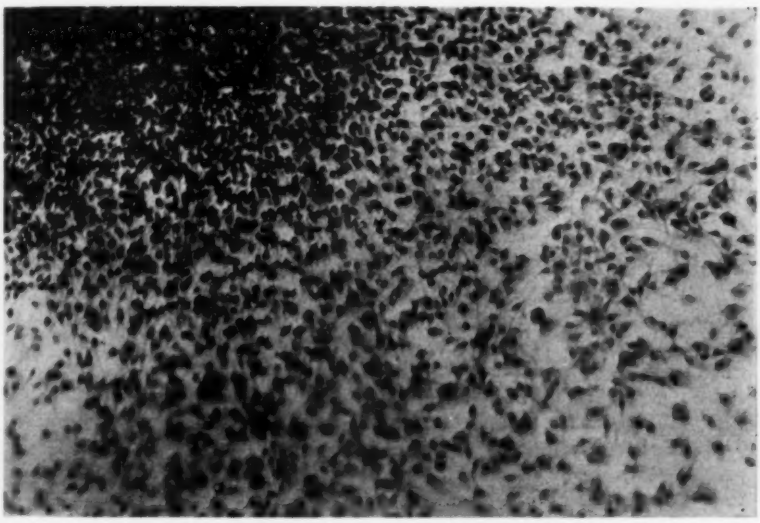

FIG. 2. PHOTOGRAPH OF STAINED CULTURE OF WALKER RAT SARCOMA 319 IN PURE STRAIN SHOWING A PORTION OF THE CENTRAL AREA OF ONE OF THE COLONIES AND THE INTERMEDIATE AND PERIPHERAL MIGRATORY ZONES

Many dividing cells, some abnormal, are present throughout the colony, even in the central area. Photograph taken directly through the wall of the metabolism cylinder. Mag. ×100.

garding the exact histogenesis of this sarcoma. It was found in 1929 arising as a tumor of the soft parts in the perineum of a female rat of the Philadelphia Albino Rat Colony. Until June 2, 1932, it was carried by transplants in rats and since then it has been grown for over seven and one-half years to date (Nov. 1939) in a pure strain in this laboratory. Some of the important cytological characteristics

of the cells of this tumor as observed in primary explants were described first by Schopper (21). Recently a brief description of the living cells of our tissue culture strain was given by Lewis (14). It should be sufficient to state here that the living sarcoma cells can be identified readily as malignant cells.

METHODS

Several methods have been devised for the study of the gaseous exchange of cells grown in tissue culture. Laser (12) modified the indirect method of Warburg for a continuous study of cell metabolism whereas Lipmann (15) used the direct method of Warburg in his work. Tiedemann and Fischer (24) developed a complicated method for the determination of carbon dioxide production based upon thermal conductivity. Victor and Lewis (26) used a very sensitive volumetric apparatus. For our work it was decided to construct an apparatus in which adequate amounts of tissue could be utilized not only for determinations of gaseous metabolism but also for chemical analyses of the tissue culture media. It was necessary, in addition, to avoid as much as possible thermal and mechanical disturbances to the cells when the cylinders supporting the colonies were transferred from the roller tubes to the metabolic apparatus. Control of these factors was achieved in the following apparatus (fig. 3). The essential feature is that the cells were grown on a small cylinder (1) which was fitted into a roller tube for cultivation and which could be transferred quickly to the metabolic chamber (2). A photograph of the cylinder with the colonies attached and a photomicrograph of the growing cells taken through the wall of a cylinder are shown in figures 2 and 4. This cylinder (1) with the cells on the inner surface was placed in the tubular portion (2) which extends from the bottom of the Dixon-Keilin flask (3). In order to obtain adequate circulation of fluid, an all glass pump (4) was placed in a side tube. This pump was operated by an external magnet controlled by a rotary contact operating at a speed of forty revolutions a minute. When the pump was in operation there was sufficient action to circulate the fluid from the lower chamber to the upper one at such a rate that the fluid ran down the surface of the flask in a thin stream. At the time of an experiment, the cylinder (1) from the roller tube was transferred quickly to the metabolic chamber (2), the plunger was inserted and the flask was connected to the manometer (5). A similar flask without tissue was attached to the opposite arm of the manometer. Thus thermal and mechanical disturbances were avoided by the quick transfer of the tissue from the growing chamber to the metabolic flask and by the absence of unnecessary shaking or scraping which would loosen the colonies.

Details of the procedure for the use of the Dixon-Keilin apparatus have been described so well (Dixon (6)) that only those features which are novel to the pumping system will be given. In order to determine if this system was adequate for the maintenance of gaseous and thermal equilibrium, several types of control experiments were conducted. After the apparatus had been connected and placed

in the water bath, the pumps were started and allowed to run for twenty-four hours in order that the magnitude of the pressure change without tissue present in either side might be observed. In the early experiments slight irregular movements were found in the manometer fluid during the first two hours of the experiment. It became obvious that these changes were due to inadequate temperature control at the start of the determination. By the use of solutions warmed to 38°C. and by the passage of air heated to the same temperature as that of the water bath through both flasks for ten minutes before the experiments, these slight changes were eliminated. To determine the efficiency of the release of carbon dioxide from

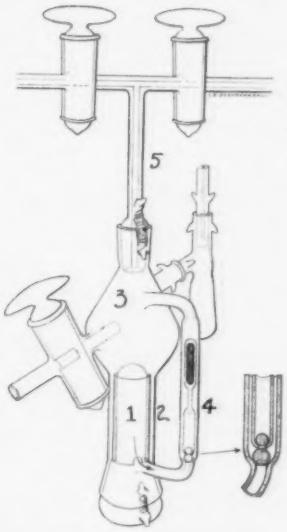

FIG. 3. THE APPARATUS FOR THE DETERMINATION OF THE RESPIRATORY METABOLISM OF TISSUE CULTURES

the solutions and the completeness of its absorption by the potassium hydroxide, tests were made in which hydrochloric acid was spilt from the side tube of one flask into bicarbonate buffered Ringer's solution. After the readings had become constant, the side stopcocks were turned allowing the alkali to come into contact with the filter paper. In these tests the fluid in the manometer arms returned to its original level, showing thereby that the absorption of carbon dioxide was complete. Another test of the same factor was made by weighing out a small quantity of sodium bicarbonate in a capsule and placing this capsule in the side arm of one flask in such a position that the capsule with its contents fell into acidified Ringer's solution when the side arm was turned. This determination resulted in the

recovery of 97 per cent of the theoretical amount of carbon dioxide liberated from the bicarbonate. A test was made also of the usefulness of the apparatus for the determination of the utilization of oxygen. Ascorbic acid and a trace of cupric ion in water were placed in one chamber and water was placed in the second vessel. A steady rate of utilization of oxygen was obtained in this experiment. These tests convinced us that the pumping system was adequate to equilibrate the gaseous with the liquid phase in the chambers.

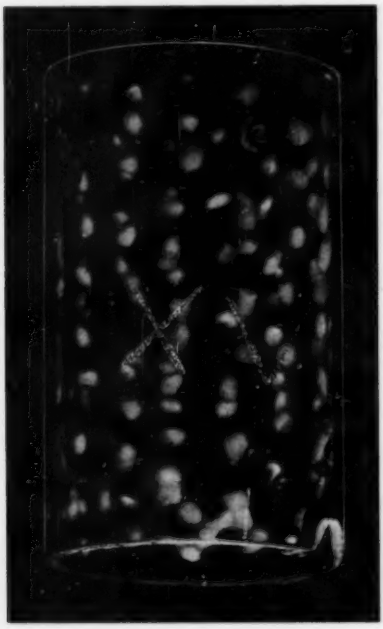

FIG. 4. METABOLISM CYLINDER WITH LIVING TISSUE CULTURE COLONIES. MAG. ×2

Experiments were undertaken then with tissue cultures. The bath was kept at a constant temperature of 37.80° ± 0.02°C. The fluid used was 0.1 per cent glucose in phosphate buffered Ringer's solution made according to the directions given by Gey and Gey (8) and sterilized by filtration. All glass vessels were sterilized by dry heat before each experiment.

The cultures were grown on glass cylinders 42 mm. long and 22 mm. inside diameter (fig. 4). These cylinders were placed in large roller tubes with a medium composed of 40 per cent heparinized chicken plasma, 25 per cent human placental

serum or in some experiments fresh horse serum, 10 per cent bovine embryo extract, and 25 per cent balanced salt solution. In some of the experiments the medium contained 40 per cent serum instead of 25 per cent. In all instances only a very minimum of plasma clot was placed on the culture cylinders to hold the colonies. The supernatant fluid was of the same composition as the clot medium but without the plasma. After four to ten days of growth the tumor colonies were transferred to the metabolic chamber and the experiment started. In the longer experiments a few of the colonies fell away from the cylinder and collected at the bottom of the flask. Because these colonies did not clump it was felt that the change in position did not affect their metabolic activity. Viability tests were made on cultures after six hours in the flask and it was found that the cells were growing normally twenty-four hours later. Bacterial tests were not made as oxygen comsumption decreased with time. Bacterial contamination, therefore, could not have been a factor for bacterial growth would have accelerated oxygen utilization.

The glucose, lactate, urea and non-protein-nitrogen determinations were done on the standard medium in which the cells were grown. The composition of this medium has been given above. The results of these determinations represent changes taking place in the medium during normal tissue culture growth under aerobic conditions. The majority of the results are expressed in terms of concentration per 100 ml. of fluid and not in absolute amounts of material gained or lost per mgm. dry weight of tissue. This concentration is compared with that in the medium of a control tube incubated without cells under the same conditions. The reason for this method of presentation is that the tissues could not be sacrificed in every experiment; and, therefore, the dry weight could not be obtained. In order to make the experiments with cells approximate each other, however, the same number of drops of fluid were placed in each tube and a comparable number of colonies were used. The observed changes in concentrations have the same significance as, for example, a blood sugar determination in the intact animal.

The glucose determinations were made by the Shaffer-Hartmann (22) method following removal of proteins by the zinc precipitation method of Somogyi (23). In a few analyses fermentable reducing substances were determined by the use of yeast fermentation. Lactates were determined by the gasometric method of Avery and Hastings (1) and checked by comparing the result of a determination by the West (30) modification of the Friedemann, Contonio and Shaffer (7) bisulfite method. The gasometric method gave 136 mgm. per cent while the bisulfite method gave 130 mgm. per cent lactic acid on the same sample of medium. Urea was determined also gasometrically (Van Slyke (25)) and non-protein-nitrogen was assayed by Nesslerization after digestion by Wong's procedure (33).

In the determination of oxygen consumption, the results are reported in terms of $mm.^3$ O_2 utilized by one mgm. dry weight of tissue per hour. The weight of tissue was obtained at the end of each experiment by collecting the colonies in a centrifuge tube, washing them once in distilled water, centrifuging, drying for twenty-four hours at 100°C., cooling in a desiccator and weighing. In some of the experiments in which chemical determinations were made, a similar procedure was used.

In the later experiments, volume determinations were made in which the cells were centrifuged into specially designed hematocrit tubes. The method of determining volume is theoretically superior to that of determining wet or dry weights as it avoids washing which process may affect the weight of the cells. Because the cells were cultured in a fluid medium, an individual colony contained only the cell mass. The volume readings, therefore, were uncontaminated by fibrin. Colonies maintained in plasmatic medium were measured after digestion of the fibrin clot with 0.3 per cent trypsin solution. This and even higher concentrations do not injure the cells.

RESULTS

In order to obtain the approximate relationship between growth and duration of cultivation, the volume of the cells was determined in different cultures over various periods of time following their place

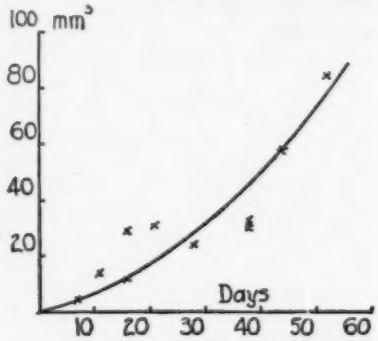

FIG. 5. RELATIONSHIP BETWEEN DAYS OF GROWTH AND VOLUME OF COLONIES OF WALKER RAT SARCOMA 319

ment in roller tubes. The initial volume in each tube was approximately the same, averaging 25 mm.³ with a variation from 22 to 30 mm.³. The increments of growth were determined by subtracting the initial volume from the final one. The results are given in graphic form in figure 5. The volumes may be converted into weights by multiplying them by the specific gravity, 1.033.

During the course of these observations many wet and dry weights of tissue culture colonies were determined. A few determinations were made also of the specific gravity of these cells. The average proportion of the weight of the dry material to the wet was 11.7 per

cent in 45 determinations. Although the majority of the results fell between 9.0 and 14.0 per cent, a few were outside these limits; and the total series varied from 6.1 to 18.2 per cent. The ratio of wet weight to volume averaged 1.033 in four determinations with a variation from 1.028 to 1.043.

TABLE 1

The consumption of oxygen by Walker Sarcoma 319

DATE, 1938	TIME OF EXPERIMENT	Q_{O_2}	DRY WEIGHT
	hours	*mm.³ O₂/mgm. dry wt./hour*	*mgm.*
Feb. 8	21.60	−3.3	2.8
14	24.00	−5.1	2.7
18	25.00	−5.7	2.6
24	5.45	−4.1	2.6
28	24.00	−4.1	3.3
Mar. 11	12.00	−4.5	2.6
Average		−4.5	

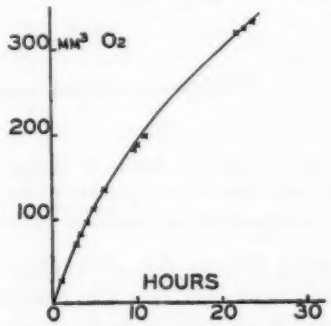

FIG. 6. OXYGEN CONSUMPTION OF WALKER RAT SARCOMA 319 FOR A PERIOD OF 24 HOURS

Data from experiment of February 28th

The results of the experiments on oxygen consumption are given in table 1. In the experiments varying in duration from 5.45 to 25.00 hours, the O_2 consumption averaged −4.5 mm.³ O_2 per mgm. tissue per hour with variations from −3.3 to −5.7. The rate of oxygen consumption decreased as the experiment progressed (fig. 6). After the cells had been ten hours in the glucose Ringer's solution, during

which time the rate of oxygen utilization was almost linear, the velocity decreased and became slower.

The utilization of glucose by this tissue growing in the roller tubes is given in figure 7. In order to determine the rate of glucose utilization, it was necessary to make many observations at different intervals on different colonies and to plot the results on the same scale. There is sufficient uniformity in the results to justify drawing a curve through the points obtained from media of the same initial concentration. The greatest fall in glucose concentration occurred in the first day and was followed by a more gradual removal of all the remaining

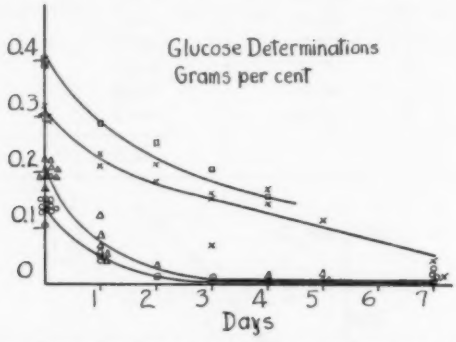

FIG. 7. GLUCOSE CONCENTRATIONS OF MEDIA IN WHICH THE COLONIES WERE GROWN FOR PERIODS OF ONE TO SEVEN DAYS
Initial concentrations are placed on the ordinate

sugar. It is interesting to note that with initial glucose concentrations of two hundred mgm. per cent the complete removal of the carbohydrate occurred in approximately the same time as with initial concentration of one hundred mgm. per cent. Increasing the initial concentrations to three or four hundred mgm. per cent, however, did not affect the velocity of utilization of carbohydrate. In a few determinations yeast fermentation was carried out on the media. Two determinations revealed the presence of 10.0 and 16.2 mgm. per cent non-fermentable reducing substances. In one experiment, a similar test was made after the sugar concentration had reached a level of 8.6 mgm. per cent and it was found that this residue was non-fermentable material. The cells had abstracted all of the fermentable material from the medium. The result of two series of

determinations in which the weights of the tissue were determined are given in table 2. The glucose utilized is calculated in terms of mgm. used per mgm. dry weight of tissue per day.

TABLE 2

Results of two series of glucose determinations on media in which Walker Sarcoma 319 was grown

GROWTH	GLUCOSE CONCENTRATION			WEIGHTS		GLUCOSE UTILIZATION*
	I Initial	II Final	Difference I − II	Wet	Dry	
days	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm.*	*mgm.*	*mgm /mgm. dry weight/day*
1	300	233	67	15.0	1.8	0.37
2	300	218	82	14.9	1.5	0.27
3	300	160	140	16.5	2.4	0.19
1	295	210	85	10.2	1.1	0.77
2	295	180	115	9.2	1.0	0.58
3	295	155	140	8.9	0.8	0.58

* Calculated on the basis of 1.0 ml. of fluid media present in each tube.

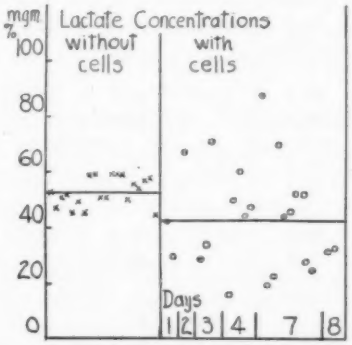

FIG. 8. LACTATE CONCENTRATIONS OF MEDIA IN WHICH COLONIES OF WALKER RAT SARCOMA 319 WERE GROWN FOR PERIODS OF ONE TO EIGHT DAYS

First column contains the results of the control experiments in which the media was incubated without cells. The second column gives the results when cells were present. This section is subdivided into days of incubation. The horizontal lines in both sections represent the average of the determinations in the control and experimental groups.

The results of the lactate determinations are given in figure 8, in which the lactate concentration in the cell medium is compared

with the control concentration after varying intervals of growth. Although the variation in the culture tubes was more than in the controls the general average is not markedly different in the two series.

TABLE 3

Non-protein nitrogen determinations on media in which Walker Sarcoma 319 was grown

DATE, 1939	GROWTH	EXPERIMENTS				DIFFERENCE II − I	PER CENT CHANGE
		I		II			
		Control	Average	With cells	Average		
	days	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*		
Mar. 14	6	18.7 18.9	18.8	22.5 23.2	22.8	4.0	21.2
16	6	18.8 17.8	18.3	22.0 22.0	22.0	3.7	20.2
29	5	23.4 21.2	22.3	34.8 36.0	35.4	13.1	58.7
Apr. 5	4	14.6 15.0	14.8	23.5 23.5	23.5	8.7	58.7
12	4	18.5 18.7	18.6	25.0 25.0	25.0	6.4	34.4
18	6	19.1 19.5	19.3	28.6 28.4	28.5	9.2	47.7
22	4	19.0 19.4	19.2	28.9 28.1	28.5	9.3	48.3

TABLE 4

Urea determinations on media in which Walker Sarcoma 319 was grown

DATE, 1939	GROWTH	EXPERIMENTS		DIFFERENCE II − I
		Control I	With cells II	
	days			
Feb. 21	3	2.78	2.78	0.00
28	6	2.26	2.26	0.00

The non-protein-nitrogen and urea determinations are given in tables 3 and 4. In each case the non-protein-nitrogen content of

the medium increased over the control concentration whereas the urea content remained unchanged in fluid incubated with cells for three and six days, the latter period being long enough to allow some cell injury.

DISCUSSION OF RESULTS

The scatter of the determinations relating to the rate of growth is so great that a definite mathematical formulation may not be given. There is a rough relationship (fig. 5) showing a steady increase in in the amount of tissue with time, forty days of growth being necessary to double the volume of cells from an initial volume of 25 mm.^{3}. This rate is in marked contrast to the rapid rate of multiplication of cell colony area described by Parker (19) for chick fibroblasts; these colonies in tissue culture double their area every two days. The ratio of dry to wet weights shows also considerable variation. Cells growing under constant conditions should have approximately similar amounts of water present. With cells imbibing fluid by pinocytosis (Lewis (13)), with the spaces between the cells varying in different colonies and with the method used, however, it is not surprising that some variations should occur. These factors affect also the determinations of the specific gravity of the cells.

Following the introduction of the Warburg and Barcroft methods of measuring the metabolism of small quantities of surviving tissue, many determinations have been made of the oxygen consumption of various types of tissue slices. In contrast, only a few determinations have been made of the respiratory activity of tissue cultures. Warburg and Kubowitz (27) reported values for the Q_{O_2} (mm.^{3} oxygen used per mgm. dry weight per hour) for fibroblasts varying from -10 to -22.9. The higher values were obtained from young cultures. These determinations were made in bicarbonate buffered serum containing 0.25 per cent glucose. Lipmann (15) obtained values of Q_{O_2} varying from -20 to -32 for heart fibroblasts and Laser (12) gave -7.8 to -25.6 for the Q_{O_2} of osteoblasts. Victor and Lewis (26) in their metabolic study of colonies of Walker Rat Sarcoma 319 reported -6.5 as the average oxygen consumption in terms of mm.^{3} per gram wet weight per min. When this figure is converted into mm.^{3} O_2 per mgm. dry weight per hour, a value of -3.3 is obtained. This value approximates more nearly the average value of

−4.5 determined in our series than the higher values reported by the other observers for different cultures. We have not had the opportunity of comparing the metabolic activity of other cultures in our apparatus with that of the Walker Rat Sarcoma 319. Our determinations and those of Victor and Lewis were made in phosphate buffered Ringer's solution whereas those of Warburg, Laser and Lipmann were carried out in plasma or in serum. It may be that there is some factor in plasma or serum which contributed to the high Q_{O_2} which these workers obtained.

In our experiments relating to lactate formation in the culture medium we did not note marked variations in the lactate concentration of the fluid even after eight days of contact with the growing tumor cells. Victor and Lewis (26) reported an average manometric increase of +7.5 mm.^{3} per gram wet weight per minute for the aerobic glycolytic activity of the same tissue and +21.2 mm.^{3} for the anaerobic glycolysis. When these values are converted into mm.^{3} of lactate per mgm. dry weight per hour the values +2.8 and +10.8 are obtained. These are very low values when compared with the results reported by Warburg (28) for tissue slices of malignant tumors. For example, the Flexner-Jobling rat carcinoma gave +25 for aerobic glycolysis and +31 for anaerobic glycolysis.

These results raise the question of the relationship of malignancy to high aerobic glycolysis. From the many experiments on normal and malignant tissues carried out in Warburg's laboratory, the generalization has been made that a tumor is a tissue with deficient respiration. The respiration of such tissue is insufficient to oxidize the intermediate metabolites and therefore lactate accumulates even when there is an adequate external supply of oxygen. The earlier work of Warburg supplied a correlation between tumor tissue, deficient respiration and high aerobic glycolysis. Later work, however, has produced many observations incompatible with this supposed relationship. Murphy and Hawkins (18) observed that certain spontaneous mouse tumors had normal respiratory rates and low aerobic glycolytic activity. Biologically there was no question that these tumors were highly malignant. Metabolically, however, they would fall into the normal group according to the Warburg classification. Hawkins (9) claimed that the ratio of aerobic glycolysis to

respiration could not be used to classify tissues in biological groups but that their anaerobic glycolytic activity might be used for such a classification. Boyland and Boyland (4) have compared recently the growth rate of various tumors with their aerobic glycolysis and respiration. With the growth rate of the tumors varying from 0.01 to 1.35 mm. increase in diameter per day, no corresponding variation was observed in respiration or glycolytic activity. Berenblum, Chain and Heatley (3) have reported determinations of respiration, aerobic and anaerobic glycolysis and respiratory quotients of normal skin epithelium and of the Shope papilloma. Identical values for these characteristics of the two tissues were found. They conclude that, when normal tissue of type similar to that of the tumor is studied, no distinctive respiratory characterization can be given the tumor tissue. They remark also that since normal tissues as brain, retina, fibroblasts, kidney medulla, synovial membrane and cartilage produce lactate under aerobic conditions and since some tumors do not, this quality cannot be used to distinguish tumor and normal tissues.

Our results with Walker Rat Sarcoma 319 provide another example of a tissue that is highly malignant but does not produce lactate aerobically. There can be no doubt that there is every opportunity for this tissue to produce lactate as it grows in the liquid medium. Other tissues growing in cultures have been shown to produce lactate. Wind (32) reports that the Rous sarcoma in Carrel cultures produces on the average an amount of lactate equal to 3 per cent of the tissue's dry weight per hour. Meier (17) records the production of 3.0 mgm. of lactate per mgm. dry weight per day by chick fibroblasts. Laser (12) gives a value of +25 for the manometric aerobic glycolysis of osteoblasts which value would correspond to 2.4 mgm. of lactate production per mgm. dry weight per day. Increments of this order of magnitude would have been detected easily had they occurred. No such changes in lactate concentration were observed, however, and Walker Rat Sarcoma 319 may be added to the list of malignant tissues which do not have a high aerobic glycolysis. Two additional factors must be considered before a final decision may be reached concerning this tumor. It is possible that in tissue culture medium the tumor may use lactate and also that the metabolic activity of a growing tissue culture may be different from that of a tissue slice. Additional

work is needed along these lines. It is obvious from these studies, however, and from the results quoted above that aerobic glycolysis or any ratio involving this factor must be used with reservations as a criterion for malignancy.

The utilization of glucose by the Walker Rat Sarcoma 319 is very rapid. For example, in one day 10.2 mgm. of tissue utilized 0.85 mgm. of glucose (table 2) or 8.3 per cent of its own wet weight. This would be equivalent to a 70 kgm. man's using 5.81 kilograms of glucose per day. Krontowski and Bronstein (11) and Holmes (10) have reported also a rapid utilization of sugar by growing tissue cultures. The question arises whether or not this sugar is stored, oxidized or converted into some intermediate product such as lactate. If 0.85 mgm. of glucose were oxidized by 10.2 mgm. of wet tissue, 635 $mm.^3$ of oxygen would be used or 533 $mm.^3$ of oxygen per mgm. dry weight per day. In our experiments on oxygen consumption an average Q_{O_2} of -4.5 was observed which would be equivalent to the utilization of 108 $mm.^3$ of oxygen per twenty-four hours per mgm. dry weight. The lack of correspondence of these figures indicated that glucose was used for other purposes besides oxidation. Since there was no lactate production, it may have been stored. Before an exact comparison may be made, however, the oxygen consumption experiments must be determined in a medium comparable to the growth medium used in the glucose determination.

In a recent study of the effect of various inhibitory agents on the growth of chick periosteal fibroblasts, Pomerat and Willmer (20) concluded that growth is connected with a direct breakdown of glucose, and not with respiration nor phosphorylation. Although their evidence was very indirect and not controlled by determinations of glucose or of oxygen consumption when the many types of inhibitors were used, it was very suggestive of the fact that glucose utilization and growth are dependent on one another. Considerable information may be obtained, therefore, concerning the energy exchange for growth by following glucose utilization by the methods outlined above.

The non-protein-nitrogen determinations are of special interest in light of the work that has been done on the nutrient requirements for cell growth. Although Baker and Carrel (2) claim that proteoses are necessary for cell growth, Willmer and Kendal (31) have demonstrated that pure proteoses in Tyrode's solution do not augment the

growth of chick fibroblasts. If plasma or embryo extract is added, however, there is a marked acceleration of the growth rate. They conclude either that there may be enzymes present in embryo juice which are able to break down the proteoses into utilizable amino acids or that there may be a particular substance in the proteoses which, when liberated, has the property of stimulating growth independently of its nitrogen supplying ability. In our work, the non-protein-nitrogen fraction of the tissue culture medium increased after contact with the growing tumor cells. This fraction is made up of nitrogen coming from urea, ammonia, amino acids, creatine, creatinine and other nitrogenous substances grouped together as undetermined nitrogen. As the urea concentration did not increase, the possibilities are that either amino acids or ammonia was responsible for the change in non-protein-nitrogen concentration in our experiments. Because the fluid medium has very little proteolytic activity, the activity must be supplied by the cells. The ability of the cells in tissue culture to digest the fibrin of plasma is well known. Demuth and Riesen (5) have studied chemically this phenomenon and report that there is a substance produced by cellular destruction which has the property of activating a ferment in the fibrin. Watchorn and Holmes (29) and Litter and his associates (16) have described a utilization of protein by tissue cultures which is decreased in the presence of glucose.

Our experiments demonstrate an increase of non-protein-nitrogen in the medium in which the cells are grown. The exact nature of the substance or substances formed, the location of the enzyme system, the effect of other substrates on its formation and many other questions must be left for future work to answer. In fact, our experiments must be considered as a preliminary survey of some of the problems connected with a metabolic study of the growth of cell colonies in tissue culture.

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SUMMARY

An apparatus was devised for the determination of the respiratory metabolism of tissue culture colonies. An average Q_{O_2} of -4.5 was found for the Walker Rat Sarcoma 319. Analyses were made of the

changes in glucose, lactate, urea and non-protein-nitrogen concentrations in the medium in which this tumor tissue was grown. A decrease in glucose concentration and an increase in non-protein-nitrogen was observed while lactate and urea concentrations did not vary significantly. The relationship of these results to each other and to the results obtained by others on tissue culture and on tissue slices is given.

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THE EXPERIMENTAL PRODUCTION OF CIRRHOSIS OF THE LIVER BY MEANS OF A DEFICIENT DIET

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During the course of an investigation into the influence of dietary factors on resistance to infection it was observed that animals that had been kept on a particular diet developed cirrhosis of the liver of a character closely resembling the Laennec type of cirrhosis in man. As far as we are aware, this is the first time that anything resembling the Laennec type of cirrhosis has been produced experimentally by an inadequate diet. It is the purpose of this paper to report briefly our present information regarding the nature and the cause of the cirrhosis.

Male albino rabbits averaging about 2500 grams in weight, and of mixed stock, were used in these studies. In no case in the present experiments were the animals subjected to any experimental infection. Each rabbit was kept in a separate cage with a large-mesh wire bottom. Water was supplied freely. All animals were weighed every week. The food intake was followed by weighing daily the amount supplied and also any uneaten residue left from the previous day.

The diet on which the animals had been kept in the first experiment in which the occurrence of cirrhosis was observed, was deficient in protein and in various vitamins. Each day the following supply of food was placed in the food cup of each rabbit: 2.7 calories of protein in the form of boiled rice and raw carrots; 118 calories of carbohydrate in the form of cane sugar and dextrin (Baker's N.F. 5 white powder), 18 calories, in addition, being derived from the carrots and rice; 94 calories of fat in the form of fresh lard. Minerals were supplied by adding to the food $1\frac{1}{4}$ grams of a salt mixture as used by Weech (1). 1 to 2 cc. of cod liver oil was given daily during the first three weeks, and 1 drop of viosterol daily thereafter. Four animals were placed

on this diet on April 22, 1939. They varied in the degree to which they ate the diet, and none of them took the full amount supplied daily. They gradually lost weight. One animal died on the 38th day after having been placed on the diet, one on the 52nd, one on the 56th and one on the 81st day. One animal was not autopsied. The remaining three showed at autopsy cirrhosis of the liver of the character to be described below.

A second experiment was carried out in which approximately the same number of calories was supplied, but with the modification that the amount of lard was reduced by one-half, the rice omitted, and the corresponding number of calories made up by increasing the amount of dextrin. No cane sugar was used in this experiment. In addition, each animal in this series was given by mouth 1 mg. of thiamin chloride (B_1) daily.[1] The other constituents of the diet were as in the first experiment. Five rabbits were placed on this diet on June 14, 1939. They ate the diet better than did the animals in the first experiment, but again with individual variations; and none of them took the full daily supply. In this group, signs of vitamin E deficiency appeared in two of the animals after the lapse of five weeks. They developed marked muscular weakness, became practically unable to move, and died. At autopsy, their muscles showed the degenerative changes described by Goettsch and Pappenheimer (2) in rabbits kept on a diet deficient in vitamin E. Beginning on the 40th day, the remaining three animals were given vitamin E by mouth (3 mg. daily of alpha tocopherol in peanut oil). In addition, from the 60th day and thereafter, vitamins of the B complex were administered daily by mouth in the following amounts: thiamin chloride (B_1), 0.5 mg.; riboflavin (B_2), 0.5 mg.; B_6, 0.2 mg.; nicotinic acid, 5.0 mg.; and instead of the original 1 drop of viosterol, 0.1 cc. of Abbott's Viosterol in Haliver Oil was given daily by mouth. Three animals in this experiment died on the 25th, 40th and 113th day respectively; one was killed on the 105th and one on the 111th day. All of them showed the cirrhosis of the liver to be described below.

[1] We are indebted to Merck and Co., Inc., for their cooperation in supplying us with the thiamin chloride, riboflavin, nicotinic acid, B_6 and alpha tocopherol used in this study; and to Dr. C. G. Mackenzie, Dr. L. Emmett Holt, Jr. and Dr. M. M. Wintrobe for their helpful suggestions regarding the rabbit's requirements of the various vitamins.

A third experiment was begun on October 23, 1939, in the attempt to gain information regarding the factor responsible for the production of the cirrhosis observed in the preceding experiments. In this experiment all animals were given an adequate supply of protein. The basic daily diet contained a total of 43 calories of protein, of which 41 calories were supplied by vitamin-free casein obtained from The Borden Co. and from Harris Laboratories, 0.7 calories by raw carrots, and 1.3 calories by boiled rice. 37 calories of fat were supplied in the form of the vegetable shortening "Crisco" (hydrogenated cotton seed oil), and 1.8 calories of fat in the carrots and rice. 120 calories of carbohydrate were provided by dextrin (102 calories), carrots and rice (18 calories). Minerals were supplied by the addition of 4 grams of the salt mixture (1). Vitamins were fed by mouth to the animals, as specified below, in the following total amounts weekly: A and D (Abbott's Viosterol in Haliver Oil) 0.7 cc.; B_1 (thiamin chloride), 3.5 mg.: B_2 (riboflavin), 3.5 mg.; B_6, 1.4 mg.; nicotinic acid, 35 mg.; E (alpha tocopherol), 21 mg. Vitamins A, D, B_1, B_2, B_6, and nicotinic acid were given in divided doses three times a week. Vitamin E was given in divided doses on days alternate to those on which the other vitamins were administered. Fifteen animals were used in this experiment. They were divided into five groups of three animals each, as follows:

Group I. Basic diet + vitamins A, D, E, B_1, B_2, B_6, and nicotinic acid. In addition, 5 grams of Fleischman's pure dry commercial brewer's yeast were mixed with the daily ration of each rabbit.

Group II. Basic diet + vitamin supplement as in Group I, but lacking yeast. Dextrose (Merck's crystalline) substituted for dextrin in basic diet.

Group III. Basic diet + vitamin supplement and yeast as in Group I, but lacking supplementary E.

Group IV. Basic diet + vitamins A, D, and E only. Dextrose used in the basic diet instead of dextrin.

Group V. Basic diet, vitamins and yeast as in Group I, except that lard was used, as in the previous experiments, instead of "Crisco" to supply the calories derived from fat.

In this experiment the animals in Groups I, III and V ate the diet well and maintained their weight with the exception of one animal

in Group III to be mentioned below. Those in Group II took the diet well for six weeks and maintained their weight, after which two gradually lost their appetites and suffered a corresponding weight loss. The third animal ate well until the last days of the experiment and lost relatively little weight. The animals in Group IV ate well for a week or two, after which their appetities became capricious and they gradually lost weight. One animal in Group IV died at the end of 40 days after several weeks of taking very little food and having lost 50 per cent of its body weight. All of the others in the experiment were killed on the 65th day. None of the animals exhibited the muscular weakness characteristic of well marked vitamin E deficiency, but at autopsy those of Groups II, III and IV showed muscular alterations of varying degree. In most cases there were only a few hyalinized fibers, but in two of the animals dead fibers were more numerous. In no case was the muscle degeneration as severe as that in animals kept on a diet completely lacking in vitamin E. Two of the animals in Group I showed rare hyalinized fibers, but no more than we have seen in control rabbits kept on a stock balanced rabbit food ("Rab-ets", from the Vitality Mills, Inc.). The other animal in this group and all of those in Group V showed no hyalinized fibers. It was apparent, on the one hand, that the animals that received no supplementary vitamin E (Group III) must have derived enough of this vitamin from the "Crisco" to prevent severe muscular degeneration, and, on the other hand, that the amount of supplementary vitamin E added to the diet of the other groups was sufficient to protect the muscles of the animals receiving yeast (Groups I and V) but was not sufficient for those not given yeast (Groups II and IV).

At autopsy, all of the animals in Groups II and IV were found to have cirrhosis of the liver. The livers of the animals in all of the other groups showed no cirrhosis, with one exception. One of the animals in Group III in which supplementary vitamin E was omitted from the diet, showed a beginning periportal connective tissue proliferation. This animal had not taken its food well and had lost one-fourth of its body weight during the experiment. The other animals in this group ate well and showed no cirrhosis. The remaining eight animals that received yeast ate the daily supply well, and none developed cirrhosis.

THE TYPE OF CIRRHOSIS PRODUCED

In each of the above experiments the cirrhosis varied in degree in different animals, and the degree of cirrhosis bore no absolute relation to the length of time that the individual animals were kept on the cirrhosis-producing diet. It is clear that there are individual differences in the rapidity with which the livers of different animals are affected. The cirrhosis in 5 of the 14 yeast-deficient animals was of marked degree (2 in Expt. I, 2 in Expt. II, and 1 in Group 2 of Expt. III); in 4 it was moderate (1 in Expt. I, 1 in Expt. II, and 2 in Group 4 of Expt. III); in 5 the cirrhosis was slight but definite (2 in Expt. II, 2 in Group 2 of Expt. III and 1 in Group 4 of Expt. III).

Macroscopically, the livers of all the animals with cirrhosis were abnormal in appearance. They were light brown or yellowish-brown. The surfaces of those least affected were smooth; those with more advanced cirrhosis were finely granular. On section there was a definitely increased resistance to the knife, and in the more advanced cases the scarring was plainly visible on the cut surface. The gall-bladders contained dark green bile, and the extrahepatic bile ducts were patent and normal in size.

Microscopically, sections taken from the different lobes showed that the cirrhosis was *diffuse*. The earlier stages appeared as an increase in connective tissue, beginning about the periportal areas and extending in bands from one lobule toward or to another. In some cases the lobules were completely surrounded by bands of newly formed connective tissue containing proliferating bile ducts and scattered mononuclear wandering cells. In other cases the scarring extended diffusely throughout the lobules, breaking them up in a completely irregular manner. A striking feature was the great *difficulty in finding necrotic liver cells*, in spite of the obvious fact that many cells must have been lost from the reduced lobules in the regions occupied by the new connective tissue. With search, isolated necrotic single liver cells and rare foci of several necrotic cells could be found, but since all stages from slight to extensive scarring are represented in our material in animals killed or dying at different periods of time between 25 and 113 days, it seems clear that the scarring was not the result of the repair of foci of necrosis, each in-

volving many liver cells. In this, the lesion resembles Laennec's cirrhosis in man in which, in spite of progressive scarring, it is ordinarily difficult to find necrosis involving more than isolated cells. Except in the type of case described some years ago by one of us (5), in which the cirrhotic process proceeds with unusual intensity from the beginning (or in which there occurs an "acute exacerbation" of the process during a previously insidious cirrhosis) and in which minute foci of necrotic liver cells are abundant, it ordinarily requires careful search to find foci of necrosis involving even one or two liver cells in human cirrhosis. In only one of our cirrhotic animals were there found some scattered foci of necrosis each involving numerous liver cells, and these necroses were obviously terminal, for the outlines of the cells and their nuclei were still visible and there was no reaction about the foci.

In three of the cirrhotic livers there was a great deal of *fat* in the hepatic cells; in six there was less fat; and in the remaining six there was no appreciable increase in fat. The livers containing most fat showed only a moderate degree of scarring, but others with a moderate or even a marked degree of scarring showed little or no increase in fat. Animals in the same experimental group on the same cirrhosis-producing diet differed markedly in the amount of fat contained in their livers.

Granules of *hemosiderin* were found in the cirrhotic livers. In most cases the hemosiderin was only slight or moderate in amount, and was situated especially at the periphery of the lobules, but in several instances it was more abundant and diffuse. The degree of hemosiderosis of the livers in these animals was entirely comparable to that in Laennec's cirrhosis in man, in which varying degrees are encountered. There was no strict correlation between the amount of hemosiderin and the degree of cirrhosis. The case showing the most hemosiderin had only a slight to moderate degree of cirrhosis, and one liver with marked cirrhosis contained only a very slight amount of hemosiderin. The amount of hemosiderin could not be correlated either with the time that the individual animals were kept on the cirrhosis-producing diets or with any dietary factor other than perhaps yeast. All of the 14 animals not receiving yeast showed deposits of hemosiderin, regardless of the other vitamin components

of their diet. Of the 9 animals receiving yeast three showed very minute amounts of hemosiderin, one a moderate amount, and 5 none at all.

In 6 of the cirrhotic livers there were inspissated plugs of bile in somewhat dilated ducts in the periportal spaces and, interestingly, in two of these, microscopic laminated *gall stones* were found in several ducts in the sections. No macroscopic gall stones were found in the gall-bladder or extrahepatic bile ducts in any of the animals. In the above-mentioned 6 cases showing plugs of bile in the intrahepatic bile ducts it is possible that some of the scarring may have been due to the biliary obstruction. In the remaining 8 cases, however, the scarring appears to have been completely independent of biliary obstruction, for the microscopic ducts were not dilated and no plugs of bile were present. The serum of nine of the 14 animals with cirrhosis was examined for bilibubin, and a plasma *jaundice* was present in six instances. In only 3 of these were plugs of bile found in the periportal bile ducts in sections.

In 7 of the animals with cirrhosis *ascites* was present. The amounts were 8, 12, 13, 15, 25, 30 and 70 cc. The animals with 8, 25 and 70 cc. had been on the low protein diet and their total plasma protein just before death was 3.5, 4.4 and 4.1 grams per 100 cc., respectively. At the beginning of the experiment their plasma protein was 6.4, 5.5 and 5.7 grams per 100 cc. respectively. The remaining animals with ascites were from Experiment III in which the diet contained ample protein. Determinations of plasma protein were not made on these animals. The amount of ascites was, in general, proportional to the degree of scarring. The *spleens* were only slightly enlarged, or normal in size. *Blood studies* in these experiments were incomplete, and no conclusions can be drawn from them. It may be stated that hematocrit readings were made of the blood of 5 of the animals with cirrhosis in the last experiment and they were not significantly abnormal. Smears of the blood of these 5 animals showed at the most only a slight anisocytosis. Smears from one cirrhotic animal in Experiment II, however, showed marked abnormalities in the size and shape of the red cells.

Coccidia were found in the livers of 4 of the 14 animals with cirrhosis. The infection was in no case severe, and the lesions were quite different

from the cirrhotic process described in this paper. Four of the 9 control animals that received yeast and did not develop cirrhosis were similarly infected with coccidia.

No constant or well marked lesions have yet been found in any other organ of the cirrhotic rabbits, with the exception of the muscle degeneration, similar to that of vitamin E deficiency, in the animals described above.

DISCUSSION

In three separate experiments 15 rabbits that were kept for periods between 25 and 113 days on diets deficient in various vitamins developed cirrhosis of the liver of a type which closely resembles the Laennec type of cirrhosis in man. This condition has not been encountered in any of the many hundreds of rabbits kept in this laboratory under the same conditions but taking a complete diet.

We do not yet know precisely what factor was responsible for the cirrhosis, but the evidence points strongly to a deficiency of some factor present in yeast, apart from vitamins B_1, B_2, B_6 and nicotinic acid. This evidence may be briefly summarized.

In the first place, neither the amount nor the character of the protein (casein) in the basic diet can be incriminated, for the cirrhosis developed in the yeast-deficient animals whether on the low protein diet (Expts. I and II) or receiving an ample amount of protein (Expt. III); and, with the one exception to be discussed below, it did not develop in any of the nine animals receiving the same protein in the same amount together with yeast (Groups I, III and V of Expt. III). If protein is implicated the factor must be the lack of a type of protein present in yeast, rather than a direct injurious action of casein on the rabbit's liver.

The form of the carbohydrate in the basal diet appears to have been unimportant for cirrhosis developed in the yeast-deficient animals regardless of whether the carbohydrate was supplied in the form of cane sugar and dextrin (Expt. I), dextrin alone (Expt. II), or dextrose (Groups II and IV of Expt. III).

That the type of fat in the basic diet was not an important factor seems clear from the fact that cirrhosis developed in the yeast-deficient animals whether they were receiving lard (Expts. I and II) or vegetable

fat (Crisco) (Groups II and IV of Expt. III), and did not develop when either lard (Group V of Expt. III) or "Crisco" (Group I of Expt. III) was given together with yeast.

Since the salt supplement was the same in the groups developing cirrhosis as in those that did not, this cannot have been a factor.

As regards vitamins, the amounts of A and D supplied should certainly have been more than adequate. No microscopic lesions of vitamin A deficiency were present in any of the animals. Furthermore, cirrhosis has not been described in any of the many studies of vitamin A and D deficiency. As is known, the rabbit needs no dietary supplement of vitamin C.

While microscopic study of the muscles of all of the animals with cirrhosis showed hyaline fibres such as result from vitamin E deficiency, in most cases this was slight in amount. It seems altogether unlikely that vitamin E deficiency was responsible for the cirrhosis. In the first place, cirrhosis did not occur in any of the rabbits of Goettsch and Pappenheimer (2) which were kept on vitamin E deficient diets and developed extreme degrees of muscular degeneration. Furthermore, Dr. C. G. Mackenzie, of the Johns Hopkins School of Hygiene, has kindly allowed us to examine sections of the livers of animals that he has kept on a diet lacking in vitamin E, and showing marked muscular degeneration. There was no suggestion of cirrhosis in those animals. Finally, there was no correlation between the degree of cirrhosis and the degree of muscular degeneration in our rabbits. Some of the animals with the most marked cirrhosis had only minimal muscle changes.

It is of interest that, in Experiment III, of the 12 animals that received identical amounts of supplementary vitamin E throughout the experiment, 6 received no yeast (Groups II and IV) and all of these developed cirrhosis and all showed muscle degeneration; whereas 6 received yeast (Groups I and V) and did not develop cirrhosis, and, of these, 4 had normal muscles and 2 showed only a very rare hyalinized fibre—no more than we have encountered in control rabbits kept on a balanced commercial rabbit food ("Rab-ets"). Since all received the same amount of vitamin E, it appears that more vitamin E is required to protect the muscles of yeast-deficient cirrhotic animals than those of animals receiving yeast and having normal livers. It

is the opinion of Morgulis (7) that both vitamin E and a water-soluble factor that is present in yeast are necessary to prevent muscular degeneration in the rabbit. It is true that our animals that received both vitamin E and yeast (Groups I and V of Expt. III) did not develop significant muscle changes, whereas those receiving vitamin E without yeast did (Groups II and IV of Expt. III); but since the latter animals had cirrhosis, we are unable at present to say whether the muscle changes were due to the lack of yeast or to an adverse effect exerted by the cirrhosis on the utilization of vitamin E.

As for the vitamins of the B complex, it is unlikely that the cirrhosis was due to deficiency of B_1, B_2, B_6 or nicotinic acid, for the animals that were given daily, by mouth, all of these vitamins in amounts which should have been more than ample to supply their needs, developed cirrhosis if yeast were omitted from the diet (Group II of Expt. III).

The diet of the animals that developed cirrhosis was deficient in choline, whereas the animals that did not develop cirrhosis were obtaining choline from the yeast. This is a factor that we are studying at present. It is known that there is a tendency for fat to accumulate in the liver in animals kept on various diets lacking choline (8), but the occurrence of cirrhosis in such livers has not been reported.

All of the above considerations point toward the cirrhosis having been due to the lack of some factor present in yeast apart from vitamins B_1, B_2, B_6, and nicotinic acid. In the three experiments every one of the 14 animals that did not receive yeast developed cirrhosis, while none of the 9 animals to which yeast was given developed cirrhosis except one. This animal was one of three on a diet containing yeast but no supplementary vitamin E (Group III of Expt. III). The animal in question took the diet poorly throughout the experiment and lost one-fourth of its original weight. At autopsy there was beginning scarring at the periphery of the lobules. The other two animals on the same diet ate well and had no cirrhosis at autopsy.

While the available information indicates that the cirrhosis in these experiments was caused by the lack of some factor that is present in yeast apart from the above-mentioned vitamins, further studies will be required to determine precisely what deficiency was responsible for the cirrhosis, and experiments are in progress toward that end.

It is also desirable to learn whether other animal species are similarly susceptible. During the course of this study György and Goldblatt (3) reported finding extensive hepatic necrosis, similar to acute yellow atrophy, in some of a group of rats kept on a deficient diet. The lesion "could not be produced at will, and was not a regular occurrence even under identical experimental conditions" (the frequency of its occurrence was not stated), nor did it occur in other rats (number not stated) receiving yeast. The cirrhosis in our animals bears no resemblance to the extensive necrosis described by the above investigators. As stated above, a striking feature of the lesion in our animals was the rarity of even isolated necrotic liver cells, in the face of the extensive and progressive scarring,—a situation comparable to the condition obtaining in Laennec's cirrhosis in man. Finally, it may be mentioned that Von Glahn and Flinn (4) have reported that the addition of yeast to the diet of rabbits reduces somewhat the tendency of the liver to be damaged by lead arsenate.

Whether or not a similar dietary deficiency plays a rôle in the production of cirrhosis of the liver in man cannot, of course, be deduced from these studies, for not all animal species react in the same manner to a given vitamin deficiency. The experiments are, however, interesting in view of the frequency of cirrhosis in chronic alcoholics, many of whom, as is well known, greatly restrict their diet and develop symptoms due to vitamin deficiencies, notably pertaining to vitamins contained in yeast (B_1 (neuritis); nicotinic acid (pellagra)). Patek (6) has reported observations on patients with alcoholic cirrhosis indicating that high vitamin therapy was followed not only by improvement in the associated conditions due to deficiencies of vitamins of the B complex (neuritis, dermatitis, glossitis, anemia), but also by improvement in hepatic function.

SUMMARY

Cirrhosis of the liver, of a type resembling Laennec's cirrhosis in man, occurred in all of 14 rabbits kept from 25 to 113 days on diets supplemented by various vitamins, but lacking yeast. The present evidence indicates that the cirrhosis was due to the lack of some factor contained in yeast, but different from vitamins B_1, B_2, B_6 and nicotinic acid. Ascites occurred in 7 of the animals. Microscopic

formed gall stones developed in the intrahepatic bile ducts in two animals.

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EXPLANATION OF ILLUSTRATIONS

(Photographs by Mr. Milton Kougl)

Fig. 1. Very low magnification of cirrhotic liver of rabbit A3 (Experiment II), which had been on the diet 105 days. The irregular masses of liver tissue between the scars show only occasional necrotic cells.

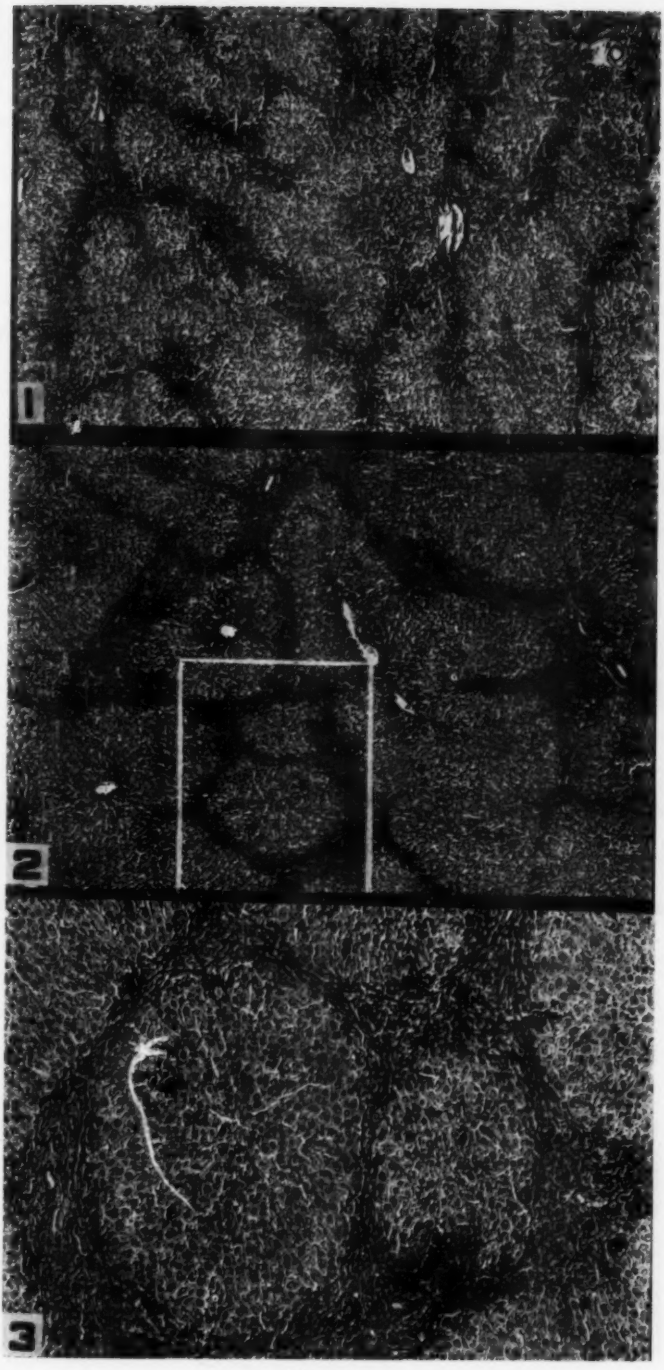
Fig. 2. Very low magnification of cirrhotic liver of rabbit B12 (Group II of Expt. III) which had been on the diet 65 days. The liver cells in the masses between the scars are well preserved, except for isolated necrotic cells. See figure 3.

Fig. 3. Higher magnification of area marked by the rectangle in figure 2. The black area at the bottom of the smaller nodule on the right is a dense area of mononuclear cell infiltration.

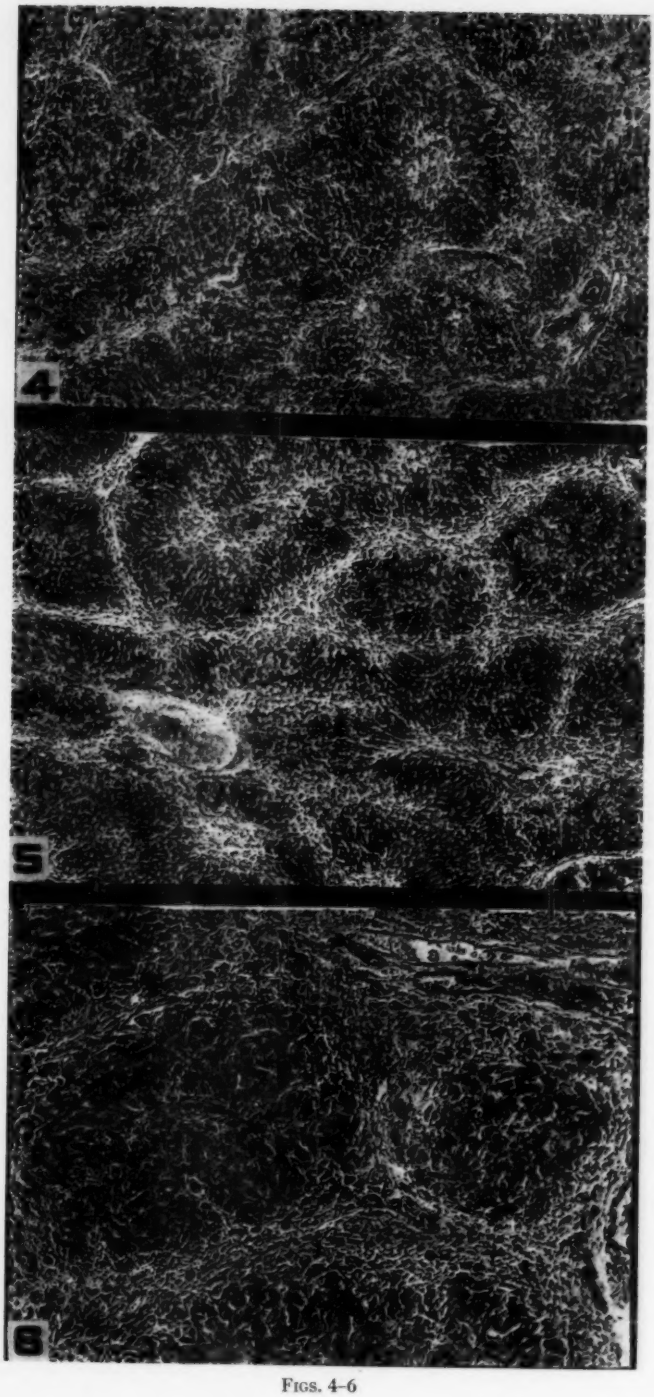
Fig. 4. Cirrhotic liver of rabbit 1 (Experiment I), which had been on the diet 52 days. No necroses in the remaining liver tissue other than of occasional, isolated cells.

Fig. 5. Cirrhotic liver of rabbit A5 (Experiment II), which had been on the diet 40 days. No necroses in the remaining liver tissue other than of occasional isolated cells. See figure 6.

Fig. 6. Higher magnification of the liver of figure 5.



FIGS. 1–3



FIGS. 4–6

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

The Compleat Pediatrician. By WILBURT C. DAVISON, M.A., D.Sc., M.D. (Second Edition) 250 pp. $4.00. *Duke University Press, Durham, N. C., 1938.*

In this edition the author has completely rearranged his material and rewritten the text. He has retained the unwieldy device of cross-reference by paragraph numbers from symptom to sign to diagnosis and vice-versa, but has rendered this system considerably less confusing by reducing the number of paragraphs from 1108 to 196. In its present condition it is a handy reference book to the entire subject of pediatrics, diseases being divided into great subdivisions, such as The Respiratory Group, The Neuropsychiatric Group, etc., with a compilation of the symptoms of each group and the diseases they occur in, followed by a short abstract of the diagnosis and treatment of each important disease. It contains numerous tables useful both for differential diagnosis and treatment, including not only the usual chart of the exanthematous diseases and excellent average growth tables, but also bone-age charts, diabetes and obesity diet schedules and a complete list of poisons. Its section on diagnostic and therapeutic procedures is concisely written and contains data nowhere else available in any one volume.

This reviewer felt that the diagnostic section of the first edition was too fragmented and confusing to be of much benefit, but that the portion devoted to therapeutics was superb. He feels that in the second edition the half devoted to therapeutics is still exceedingly well done and should be of tremendous value to practicing physicians, and that the diagnostic half, while not flawless, has been greatly improved.

A. J. S.

Modern Clinical Psychiatry. By ARTHUR P. NOYES. 2nd ed., rewritten and enlarged. 570 pp. $5.00. *W. B. Saunders Company, Philadelphia and London, 1939.*

The second edition of this textbook contains revisions which bring its usefulness up to date, including a review of the pertinent current literature. It is a really practical, straightforward presentation of the psychobiological viewpoint of man. The book deserves the attention of all medical men, as well as of students of psychiatry. The material is well organized, the presentation is simple and readable. The final and new chapter on psychiatry and general medicine, is somewhat abbreviated, doing little more than to recognize the expansion of the

psychiatrist's interest into general medical fields. Students liked the previous edition and will find in this one a fuller statement of a sound working philosophy. The bibliographies at the end of each chapter, although not extensive, are pertinent and helpful.

T. A. C. R.

Surgery of the Eye. By MEYER WIENER AND BENNETT Y. ALVIS. 445 pp. Illus. $8.50. *W. B. Saunders Company, Philadelphia and London, 1939.*

This book is an excellent concise text for students of ophthalmic surgery. It is not, nor did the authors intend it to be, a comprehensive reference book, but it does include descriptions of most of the common eye operations together with practical comments based on the authors' rich experience in this field. Careful consideration is given to such important points as preparation of the patient, anesthesia and pre- and post-operative care which are so often slighted in books of this nature. The common minor surgical procedures such as removal of foreign body in the cornea, treatment of chalazion and pterygium are given due consideration. The major ocular operations are clearly described and profusely illustrated. No attempt is made to cover all of the procedures for any given condition, but all the usual procedures and particularly those which have been most satisfactory in the authors' hands are given. There is a distinct tendency throughout the book to stress the methods which have been devised by the authors, sometimes at the expense of the alternate procedures which have been more generally accepted by the profession. Examples of this are particularly seen in the description of operations for retinal detachment and in the treatment of corneal scars. In the latter case more space is devoted to the authors' operation of corneal resection than to all the types of keratoplasty combined. The commoner procedures in plastic surgery about the eyes are described and clearly illustrated. The drawings and diagrams are plentiful and practical. What they lack in artistic finesse is made up for in diagrammatic clarity. A simple up-to-date textbook on eye surgery, in the English language, has been needed for a number of years and this book promises to fill the requirement very well.

J. M. M.

The Patient as a Person. By G. CANBY ROBINSON. 423 pp. $3.00. *The Commonwealth Fund, New York, 1939.*

Were I asked, "what has been the greatest advance in the practice of medicine during the past quarter century?", I am sure I should answer impulsively, "the realization of the importance of psychic factors in illness." Perhaps I would not be able successfully to defend this opinion, for it is vulnerable at many points and there are opposing views ready, I am sure, to attack it. Nevertheless the importance of psychic factors in medicine, even though perhaps not of the very first rank, is attested by the interest displayed in these matters by the leading teachers and practitioners of medicine. The subject is in fashion and all of them hasten to add their word to the discussion. Many of these contributions are remarkable

chiefly for their naivete. They bear evident marks of the eager enthusiasm characteristic of a new interest. A reader unfamiliar with the topic might get the impression that the recorded observations and views are something quite novel and startling. Needless to say they are not, for they are as old as medical practice itself. Nevertheless this eager enthusiasm is refreshing because it indicates a turning of attention from the too exclusive study of disease to a consideration of the human being who is ill. A term is desirable to compress and define this rejuvenated point of view. The two most frequently used are psychobiology and psychosomatic medicine. The Journal of Psychosomatic Medicine has recently been founded to serve as a forum for observations and discussions in this field.

Dr. Robinson, who had already won distinction in the fields of internal medicine and medical administration, lately turned his attention to a study of the social components of illness. The book before us summarizes observations made at The Johns Hopkins Hospital over a period of three years. Dr. Robinson approaches the subject from the unpretentious standpoint of an experienced internist with broad and warm human interests but without formal training in psychiatry. His plan has not been overly ambitious for he has restricted his study to an estimate of the importance in illness of the more usual and obvious social disadvantages and conflicts. He has made no attempt to analyze deep-seated neuroses or to lay bare the unsuspected secrets of the heart, wisely acknowledging the lack of special training and skill in the difficult search for these recondite objects. The book gains in value by limiting its scope to matters of everyday experience with which all of us are thoroughly familiar but which are usually ignored or neglected as important factors in the production of illness. It is impressive indeed to see these homely situations carefully reviewed and their significance pointed out in case after case. Especially valuable is the demonstration of the part they play in coloring the clinical picture of organic disease. To understand illness and succor the sick it is not enough to establish the presence, let us say, of rheumatic heart disease or angina pectoris and prescribe appropriate medicines. To stop there is to miss the true significance of many symptoms and the opportunity to administer remedies which often are more efficacious than drugs.

To the practicing physician this book will be valuable in drawing his attention to the importance of a simple investigation of the ordinary facts of social environment. No technical training is required to conduct the investigation, the facts lie openly before the eye. All that is needed is human interest and an appreciation of the far reaching effects of social environment in illness. But anyone concerned with medical practice or the care of the sick will find the book stimulating and serviceable. Indeed it may be read profitably by the layman and will give him a new aspect of illness which may help him to understand some of his own difficulties and to a sympathetic appreciation of those of his relatives and friends. The book is clearly written and without affectation. Everyone can read it with pleasure and easy understanding since technical terms and labored discussions are carefully avoided.

L. H.

Cardiovascular Diseases. By DAVID SCHERF AND LINN J. BOYD. 458 pp. $6.25. *C. V. Mosby Company, St. Louis, 1939.*

The purpose of this work is best explained in the following quotation from the authors' preface: "In contrast to the wealth of exhaustive volumes, there is a paucity of small books which present concise discussions of the most important diagnostic and therapeutic problems in the field of cardiovascular diseases. This book is not intended to be a textbook and does not make any claim to completeness. Rather it is intended to supply considerable practical information by brief discussions for direct application to diagnosis and treatment without recourse to complicated methods and apparatus."

The volume is relatively of small size, light in weight, well printed and, therefore, considerably more convenient to handle than the average textbook dealing with heart disease. The subject matter is treated in a conventional manner with a style which is neither especially fluent nor actually tedious. A suitable proportion of space is allotted to treatment and the therapeutic measures suggested are of a practical nature. There is no bibliography.

The book will find its greatest usefulness as a preliminary guide for medical students in the field of common cardiovascular disorders.

R. F.

Proctology for the General Practitioner. By FREDERICK C. SMITH. 386 pp. Illus. $4.50. *F. A. Davis Company, Philadelphia, 1939.*

Another book covering diseases of the lower intestinal tract. It is concise but enters into too much surgical detail for the general practitioner. The author has made many subjects—minor in pathology, but major in consequence—appear so simple and easy that there may be a tendency on the part of the non-surgical physician to attempt to carry out, in his office, the surgical treatment outlined. Actually, many such cases should be hospitalized and should be under the care of a competent surgeon or proctologist. The full chapters on pre-operative and post-operative care and the longer chapter on anesthetics—local, general, and regional—certainly do not belong in the realm of the general practitioner. He advocates, rather strongly, the injection treatment of internal hemorrhoids. This method of handling hemorrhoids should be applied in the treatment of only quite a small group of uncomplicated cases. It is not the desirable method and the results are not permanent in the majority of cases. His chapter on Lymphopathia Venereum, a subject of increasing interest to the general practitioner, is much too abbreviated. The book is adequately illustrated but has no bibliography.

The book is too brief medically and too full surgically to be of any great value to the general practitioner.

T. R. C.

Eye, Ear, Nose and Throat Manual for Nurses. By ROY H. PARKINSON. 4th ed. 243 pp. Illustrated. $2.25. *C. V. Mosby Company, St. Louis, 1939.*

An excellent nontechnical manual written primarily for class room work in teaching nurses in the training school. The subject matter is condensed and is intended to give the student nurse a general idea of what may be encountered in eye, ear, nose and throat cases in order that she may be able to follow directions given by the physician.

The manual is divided into three parts. Part I, after discussing the anatomy and physiology of the regions concerned, takes up the more common diseases, together with their care and treatment. The subject matter of each lecture or lesson is covered in a quiz at the end of the lesson. Part II takes up the technic and methods employed in the operating room. Operations are described and the instruments illustrated. Part III discusses the eye, ear, nose and throat problems encountered by the public health nurse.

The manual is well illustrated and recent advances in methods and treatment are introduced.

N. F. A.

Clinical Electrosurgery. By GUSTAVUS M. BLECH. 389 pp. Illus. $4.00. *Oxford University Press, New York, 1938.* (Oxford Medical Publications).

A very helpful feature of Dr. Blech's book is a glossary which has been inserted at the beginning, in order to define the terms which are not familiar to the average surgeon. The definitions are arranged alphabetically, rather than in accordance with any orderly presentation of the fundamentals of electrophysics.

The first chapter "Historical Development" is by a noted authority, H. A. Colwell, who has previously written on this subject. He gives a very timely outline of the history of electrosurgery. This is particularly helpful to American readers for very little has been written on the subject in this country.

In chapter 4, "General Technique", and chapter 5, "Indications and Limitations; Nomenclature", the author clarifies the very much confused electrosurgical nomenclature of the present time. For example, he suggests the word "electrotome" for the various active electrodes, whether they be blades, loops, needle-shaped, or otherwise. "Electrotomy" is suggested in place of acusection and various other words describing cutting with an electric current. "Electro-excavation" is another helpful term, to replace the phrase "scalloping out tissue with electrosurgical loop".

Chapter 6 "Radiotherapy of Neoplasms" has been written by Colwell and B. W. Windeyer. Since electrosurgery and radiotherapeutics are used so frequently in the treatment of the same patients, it is important to have them considered simultaneously.

The first six chapters deal in a general way with electrosurgery and radiotherapy. The next ten chapters deal with operations on particular regions of the body, such as skin, head, spine, thorax and abdomen. In these chapters the author quotes freely from experts in the special fields.

The entire work is written with a sensible attitude toward the surgical problems for which electrosurgery is valuable. The author is not overly enthusiastic as "some proletarian electrosurgeons" might have attempted to be in their efforts to substitute electrosurgery for the well-tried conventional instruments of surgery. In general, the author is to be complimented on this valuable contribution to medical literature. However, his book could have been made more useful by increasing the number of illustrations and the reviewer would have preferred to see simple diagrams illustrating general principles of apparatus, rather than pictures of modern commercial machines, showing the names of the manufacturers. There are so many good apparatuses that the impression should not be given that one particular type is to be preferred. I am sure the author did not intend to give this impression.

G. E. W.

BOOKS RECEIVED FOR REVIEW

Microbiology and Pathology. By CHARLES F. CARTER. 2nd. ed. 755 pp. Illus. $3.25. C. V. Mosby Company, St. Louis, 1939.

Eye, Ear, Nose and Throat Manual for Nurses. By ROY H. PARKINSON. 4th ed. 243 pp. Illus. $2.25. C. V. Mosby Company, St. Louis, 1939.

Brucellosis in Man and Animals. By I. FOREST HUDDLESON. 339 pp. Illus. $3.50. The Commonwealth Fund, New York, 1939.

Textbook of the Principles and Practice of Nursing. By BERTHA HARMER AND VIRGINIA HENDERSON. 4th ed. revised. 1047 pp. Illus. $3.00. The Macmillan Company, New York, 1939.

Nutrition and Diet in Health and Disease. By JAMES S. MCLESTER. 3rd ed., rewritten. 838 pp. $8.00. W. B. Saunders Company, Philadelphia and London, 1939.

Diseases of the Foot. By EMIL D. W. HAUSER. 472 pp. Illus. $6.00. W. B. Saunders Company, Philadelphia and London, 1939.

Attaining Womanhood. By GEORGE W. CORNER. 95 pp. $1.00. Harper and Brothers, New York and London, 1939.

Office Gynecology. By J. P. GREENHILL. 406 pp. Illus. $3.00. The Year Book Publishers, Inc., Chicago, 1939.

Introduction to Medical Mycology. By GEORGE M. LEWIS AND MARY E. HOPPER. 315 pp. Illus. $5.50. Year Book Publishers, Chicago, 1939.

Experimental Pharmacology and Materia Medica. By DENNIS E. JACKSON. 2nd ed. 906 pp. Illus. $10.00. C. V. Mosby Company, St. Louis, 1939.

Topographic Atlas for X-Ray Therapy. By IRA I. KAPLAN AND SIDNEY RUBENFELD. 120 pp. 55 plates. $4.00. Year Book Publishers, Chicago, 1939.

Primer of Allergy. By WARREN T. VAUGHAN. 140 pp. Illus. $1.50 C. V. Mosby Company, St. Louis, 1939.

Die Funktion der Nebennierenrinde. By F. VERZÁR. 266 pp. Illus. 25 Swiss francs. Benno Schwabe & Co., Basel, 1939.

Zur Differentialdiagnose seltener Skeletterkrankungen. By MAX LÜDIN. 36 pp. Illus. Paper bound, 4 Swiss francs. Benno Schwabe & Co., Basel, 1939.

Sketches in Psychosomatic Medicine. By SMITH ELY JELLIFFE. 155 pp. $3.00. Nervous and Mental Disease Monographs, New York, 1939.

Marihuana, America's New Drug Problem. By ROBERT P. WALTON. 223 pp. Illus. $3.00. J. B. Lippincott Company, Philadelphia, 1938.

Textbook of Nervous Diseases. By ROBERT BING. Translated and enlarged by Webb Haymaker from the 5th German edition. 838 pp. Illus. $10.00. C. V. Mosby Company, St. Louis, 1939.

Synopsis of Pediatrics. By JOHN ZAHORSKY. 3rd ed. 430 pp. Illus. $4.00. C. V. Mosby Company, St. Louis, 1939.

CHEMISTRY OF THE ENZYMES[1]

JOHN H. NORTHROP

The Rockefeller Institute for Medical Research, Princeton, N. J.

One of mankind's earliest observations must have been that there was a great and obvious difference between living and non-living matter. Until comparatively recently every advance in knowledge of living things seemed only to widen the distinction. In the course of the eighteenth century, however, it was found that certain reactions, previously considered to be characteristic of living things could be separated from the living organism. Thus, Spallanzani was able to cause the digestion of meat to take place outside of the animal by allowing gastric juice to act on the meat *in vitro*. This experiment was one of the first steps in narrowing the gap between the living and non-living. The observation was soon extended to many other reactions by the work of Payen and Persoz, Schwann, Kühne, Buchner and others. It gradually became evident that many, if not all of the reactions to be found in living cells were due to the presence of minute amounts of very active, unstable substances. If these substances could be separated from the cells the reaction could be made to take place *in vitro*. These hypothetical active substances which control the reactions occurring in living organisms were given the name "enzymes" by Kühne.

Berzelius, a century ago, pointed out that these substances acted in a way similar to the catalysts of the chemist and suggested that enzymes be considered as special catalysts formed by the cells. This view met with great opposition since many workers considered enzymes to differ qualitatively from ordinary chemical substances. The work of Tamman, Arrhenius, Henri, Michaelis, Nelson, Euler,

[1] Thayer Lectures presented at The Johns Hopkins Medical School, January 11th and 12th, 1940. The original reports of the work reviewed here may be found in a series of papers in the *Journal of General Physiology*, 1920-1940. The work is reviewed in detail in "Crystalline Enzymes," Columbia University Press, New York City, 1939.

Willstätter, Warburg and other chemists, however, has shown that Berzelius' viewpoint was correct and it is now generally admitted

FIG. 1. PEPSIN CRYSTALS. MAGNIFICATION × 90

that enzyme reactions are a special kind of catalysis and do not differ qualitatively from other chemical reactions.

While the study of enzyme reactions made rapid progress all at-

tempts to isolate an enzyme and so determine its chemical nature were unsuccessful until very recently. Indirect evidence pointed to the

FIG. 2. PEPSINOGEN CRYSTALS. MAGNIFICATION × 340 (HERRIOTT)

conclusion that enzymes were proteins and in 1926 Sumner isolated a protein from beans which he considered to be the enzyme, "urease," the cell catalyst which decomposes urea. Shortly afterwards pepsin

was isolated and crystallized. Since then the following enzymes have been isolated and crystallized: trypsin and its precursor, α, β

FIG. 3. PEPSIN INHIBITOR. MAGNIFICATION × 1590 (HERRIOTT)

and γ chymotrypsin and its precursor, by Kunitz and Northrop; carboxypeptidase by Anson; ribonuclease by Kunitz; pepsinogen by

Herriott; ficin by Walti; papain by Balls, Lineweaver and Thompson; amylase by Caldwell, Booher and Sherman; lysozyme by Abraham and Robinson. These enzymes all catalyze hydrolytic reactions and are, so far as is known at present, simple proteins; that is, no evidence for a special "prosthetic group" other than amino acids has been found.

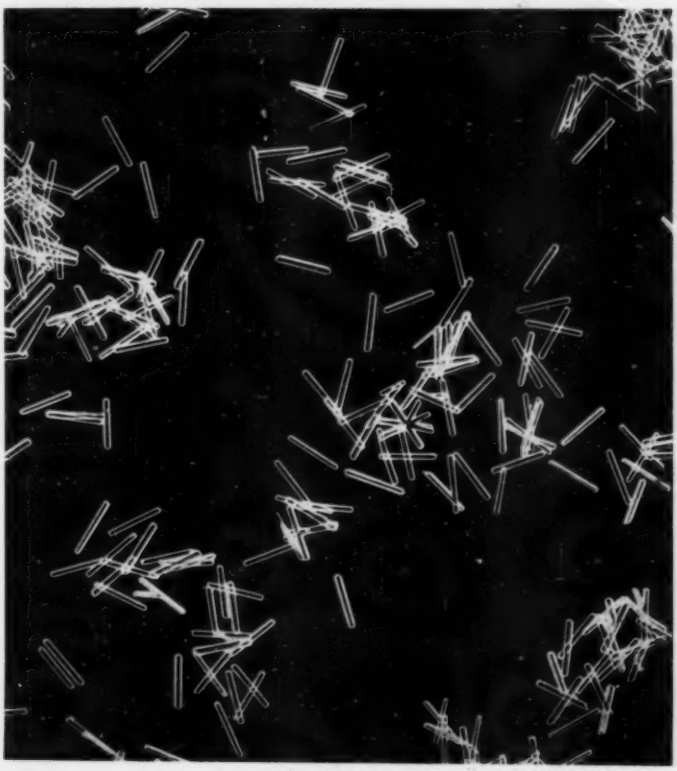


FIG. 4. TRYPSIN CRYSTALS. MAGNIFICATION × 202 (KUNITZ)

In addition to these hydrolytic enzymes, a series of respiratory ferments has been isolated by Warburg and his collaborators. Sumner and Dounce have isolated catalase, and tyrosinase has been isolated by Dalton and Nelson. These enzymes are connected with oxidation

reactions and are conjugated proteins, *i.e.*, they contain one or more prosthetic groups in addition to the amino acids.

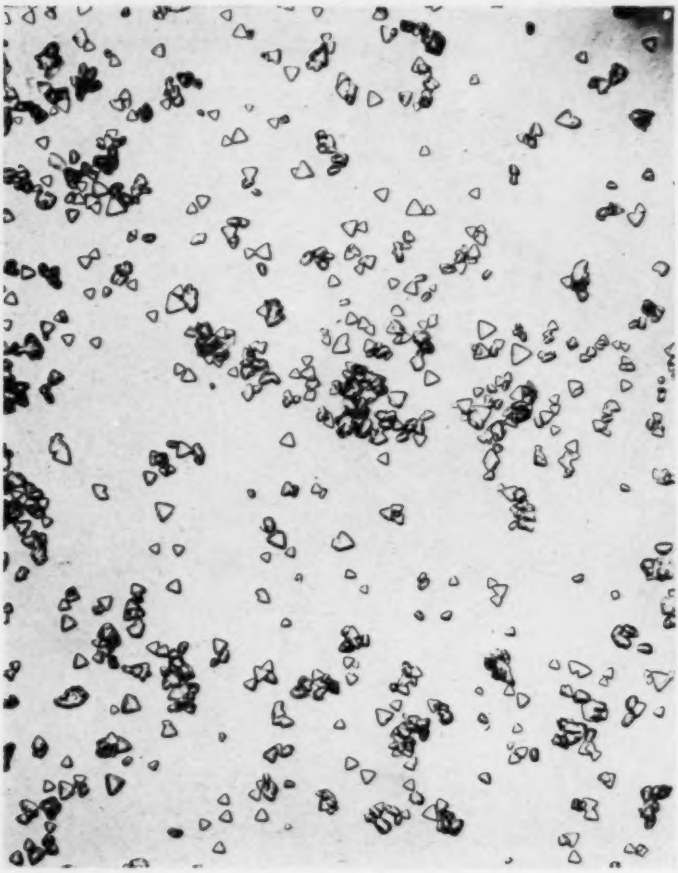


FIG. 5. TRYPSINOGEN CRYSTALS. MAGNIFICATION × 220 (KUNITZ)

It appears, therefore, that the characteristic chemical reactions occurring in living cells are caused by the presence of minute amounts

of certain peculiar proteins, so that the nature and mode of action of enzymes is now fairly well established.

FIG. 6. CHYMO-TRYPSINOGEN CRYSTALS. MAGNIFICATION × 260 (KUNITZ)

In the meantime, however, a new controversy over the nature of "viruses" has arisen, quite similar to the early controversy over the

nature of enzyme reactions. Iwanowski and Beijerinck described the first of these active agents. A large number of such filterable

FIG. 7. CHYMO-TRYPSIN CRYSTALS. MAGNIFICATION × 120 (KUNITZ)

viruses were soon described, among them the bacteriophage of Twort and d'Herelle which possesses the remarkable property of dissolving

bacteria. These filterable viruses increase in quantity in the presence of living cells and for this reason have been considered by many

FIG. 8. CHYMO-TRYPSIN. β- CRYSTALS. MAGNIFICATION × 315 (KUNITZ)

workers to be themselves living organisms capable of synthesizing themselves from simpler compounds.

On the other hand, Doerr, Bordet and others have pointed out that the facts may be accounted for by the much simpler assumption that

FIG. 9. CHYMO-TRYPSIN. γ– CRYSTALS. MAGNIFICATION × 15.5 (KUNITZ)

the virus is a product of cell metabolism. From this point of view the viruses are enzymes which stimulate the cell to form more virus

or which form themselves from an inactive precursor contained in the host cell, as do some enzymes.

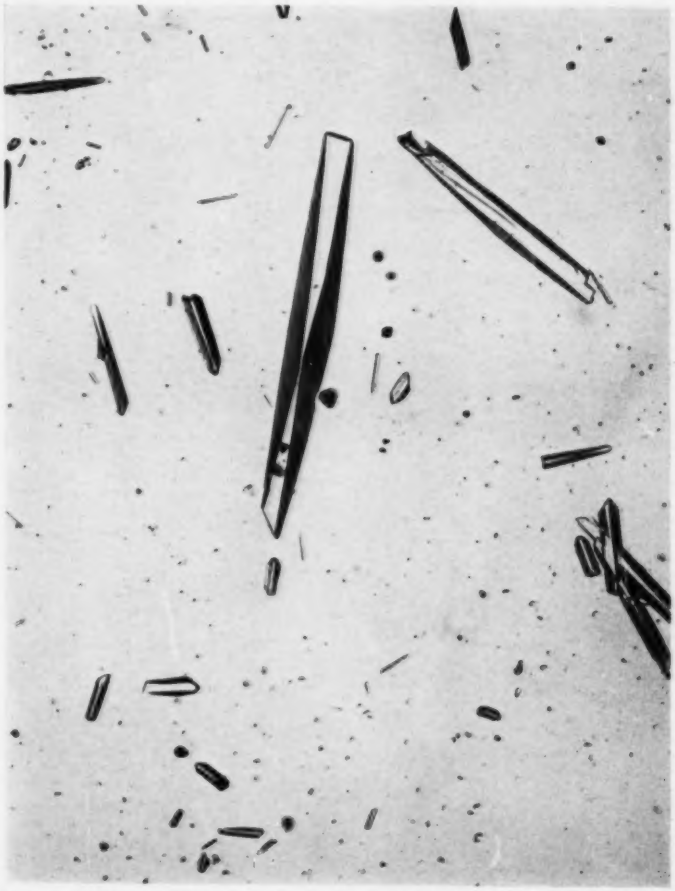


FIG. 10. CARBOXYPEPTIDASE CRYSTALS. MAGNIFICATION × 85 (ANSON)

Stanley, and Bawden and Pirie have isolated and crystallized a series of nucleoproteins which have the properties of various plant

viruses, and Schlesinger, Bronfenbrenner, and the writer have obtained nucleoproteins which have the properties of bacteriophage.

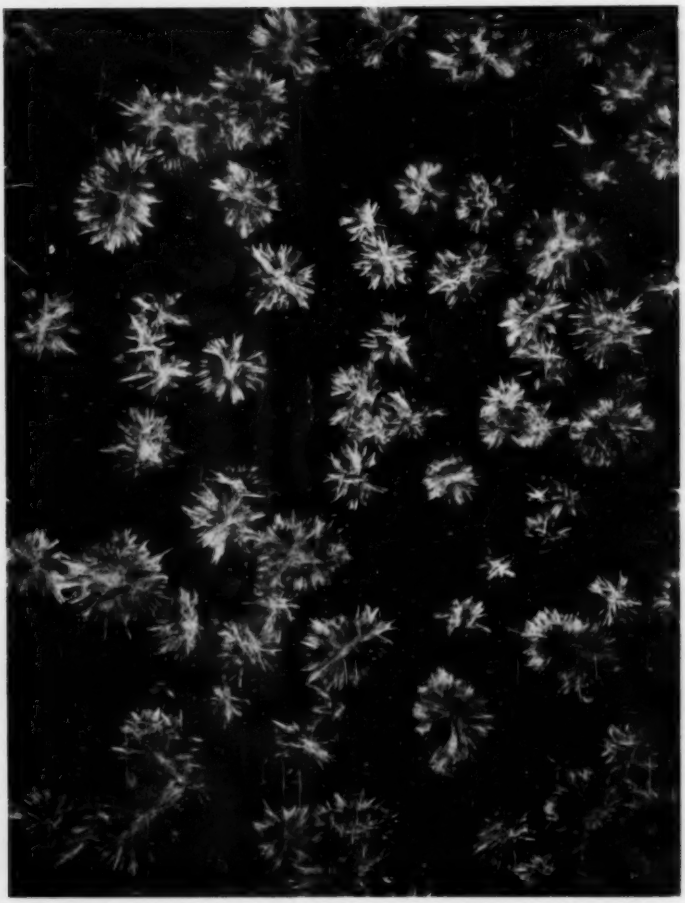


FIG. 11. RIBONUCLEASE. ROSETTE CRYSTALS. MAGNIFICATION × 190 (KUNITZ)

In chemical composition, therefore, the viruses are closely related to the enzymes.

ISOLATION AND CRYSTALLIZATION OF PEPSIN, TRYPSIN, CHYMO-TRYPSIN AND THEIR PRECURSORS

Unfortunately experience has shown that no one method can be relied upon to lead to the isolation and crystallization of enzymes. The most that can be said is that they are best handled by the technique of protein chemistry and that large quantities must be used so that weighable solid precipitates are available and not simply dilute solutions.

In the work reported here this has been the guiding principle, and several grams or, better several hundred grams, of each step in the process of purification have been at hand before proceeding to the next step.

Pepsin

Pepsin was isolated and crystallized from commercial pepsin preparations by precipitation with magnesium sulfate in dilute acid. It has also been obtained from bovine gastric juice. Active pepsin exists only in the gastric juice and is present in the gastric mucosa as an inert protein, pepsinogen. Pepsinogen has also been obtained in crystalline form by extraction from the mucosa in slightly alkaline solution and fractionation by means of ammonium sulfate.

Pancreatic Enzymes

Digestion of proteins in the small intestine is caused by the pancreatic juice, and it was thought at first that this juice contained only one proteolytic enzyme called by Kühne "trypsin." It is now known, however, from the work of Willstätter, Waldschmidt-Leitz and others that pancreatic juice contains a series of proteolytic enzymes, erepsin, aminopolypeptidase, carboxypolypeptidase, protaminease, chymotrypsin and probably others. Like pepsin, the enzymes in the pancreatic juice became active only after secretion and are present in the gland itself in the form of inactive precursors. Trypsin was originally isolated and crystallized from activated pancreatic extract by repeated fractionation with ammonium sulfate. The procedure was difficult, however, and a much more efficient method was worked out by which the inactive precursor of trypsin (trypsinogen) was isolated from inactive acid extracts of fresh pancreas. The inactive precursor

of chymo-trypsin was also isolated from such extracts, as well as a substance which powerfully inhibits trypsin activity. Anson isolated and crystallized carboxypeptidase from activated pancreatic extract and was able to show that this enzyme also exists in fresh pancreas in an inactive form. Conditions for the transformation of the inactive precursors to the active enzymes have been worked out and the active enzymes themselves crystallized.

Chymotrypsin on standing under certain conditions is transformed into two new enzymes, β and γ chymotrypsin. These new enzymes have slightly lower molecular weights than the parent enzyme and very nearly the same activity. It is probable that a portion of the protein molecule which has little connection with the structure responsible for the activity is split off. The three chymotrypsins crystallize as solid solutions under certain conditions but form mixtures, which may be separated under other conditions (Kunitz).

Kunitz has recently isolated and crystallized an enzyme, ribonuclease, which hydrolyzes nucleic acids. It is remarkable in that the molecular weight appears to be only about 18,000.

PURIFICATION OF BACTERIOPHAGE

A nucleo protein has been isolated from lysed staphylococci cultures which has the properties of bacteriophage, *i.e.*, it increases when inoculated into a growing culture of staphylococci and eventually causes complete dissolution of the bacteria. The protein is practically homogeneous in the ultra-centrifuge and has a sedimentation constant of about 650×10^{-13} cm. dyn^{-1}, sec^{-1} corresponding to a molecular weight of about 200 million. The minimum quantity of the nucleoprotein which can be detected is about 5×10^{-16} grams. If this quantity is assumed to be one molecule then the gram molecular weight of the substance is about 300 million grams. This is about the same order of magnitude as the molecular weight by the sedimentation velocity method. Diffusion measurements in concentrated solution show that the protein and the active agent diffuse at the same rate and give a value for the molecular volume which agrees approximately with that found by the centrifuge method. In extremely dilute solution the phage diffuses more rapidly, indicating that the phage particle may dissociate into smaller units as do the hemocyanins. Similar

results have been reported by Bronfenbrenner. The phage protein has not been crystallized.

TESTS OF PURITY

There is no doubt that the crystalline materials described above are proteins but the proof that these proteins are the enzymes themselves is open to question owing to the difficulty of establishing the purity of a protein. In organic chemistry constant properties and composition through repeated fractional crystallization are considered as proof of purity. In the case of proteins, constant analysis means very little and the melting point, which is the most sensitive test, cannot be used since proteins decompose instead of melting. However, the solubility of the protein, which is theoretically analogous to the melting point, may be determined. When this criterion is used, however, all proteins so far studied, whether enzymes or not show more or less deviation from the theoretical results expected for a pure substance. In the case of trypsin, chymotrypsin, pepsin and ribonuclease the deviation is small and is of the order of magnitude of that observed with other proteins such as hemoglobin and egg albumin. Chymotrypsinogen approaches very closely the theoretical curve and is one of the purest proteins ever obtained. In the case of pepsin it appears from recent experiments of Herriott and Desreux that there may be several active proteins since preparations from different sources give proteins of slightly different activity, stability and solubility.

The homogeneity of proteins may also be tested by measurements of the rate of sedimentation in the ultracentrifuge described by Svedberg and by the rate of migration in the electrophoresis cell developed by Tiselius. Pepsin preparations which are homogeneous by these tests, however, have been found to contain two or more components by the solubility method. This latter method, therefore, appears to be the most reliable test of purity.

RELATION OF THE ACTIVITY TO THE PROTEIN

It may be shown, in addition to these tests of purity, that loss in activity is accompanied by changes in the chemical properties of the protein. Thus, when phage or the enzymes are heated the activity is lost and at the same time denatured protein is formed. In the case of

trypsin and chymotrypsin the reaction is rapidly and completely reversible so that these enzymes exist in equilibrium with an inactive, denatured form. This equilibrium is accurately expressed by van't Hoff's equation connecting the heat of reaction and the effect of temperature on the equilibrium constant and gives a value for the latter of 67,000 calories per mol. The equilibrium between native and denatured trypsin is also affected by the pH of the solution, and this accounts in part for the effect of the pH on the activity of the enzyme. In the case of pepsin the denatured pepsin may also be reconverted to native active pepsin.

The relation between protein and activity may be further tested by digesting the protein with another enzyme. If trypsin or chymotrypsin is hydrolyzed by pepsin there is a loss in activity corresponding to the destruction of the protein. Digestion of phage with chymotrypsin leads to the formation of insoluble inactive protein. If the rate of diffusion of the protein and the activity is determined it is found that the diffusion proceeds at the same rate when followed by either method. The active molecule and the protein molecule therefore diffuse at the same rate.

Gates showed that the efficiency of various wave-lengths of ultraviolet light on the inactivation of pepsin is the same as that for the relative absorption at various wave-lengths by pepsin solutions. The result indicates that each quantum absorbed by a protein molecule inactivates the molecule. Similar results were obtained with bacteriophage.

These experiments indicate strongly that the activity is a property of the protein molecule itself but unfortunately furnish no information as to the chemical structure in the molecule responsible for its activity. Owing to the extreme complexity of the protein molecule it is impossible, at present, to determine the structural configuration of the whole molecule, but some information in this connection has been obtained by studying the known changes in the molecule which affect the activity. The most definite of these reactions is the formation of acetyl derivatives of pepsin. Two crystalline acetyl derivatives have been obtained by the action of ketene in aqueous solution at pH 5.0-6.0. The first compound contains 3 or 4 acetyl groups attached to the primary amino groups. It has the same activity as the original pepsin.

A second derivative containing 7 acetyl groups has also been obtained. It has 60 per cent of the activity of the original pepsin. On standing in acid this compound loses half of its acetyl groups and at the same

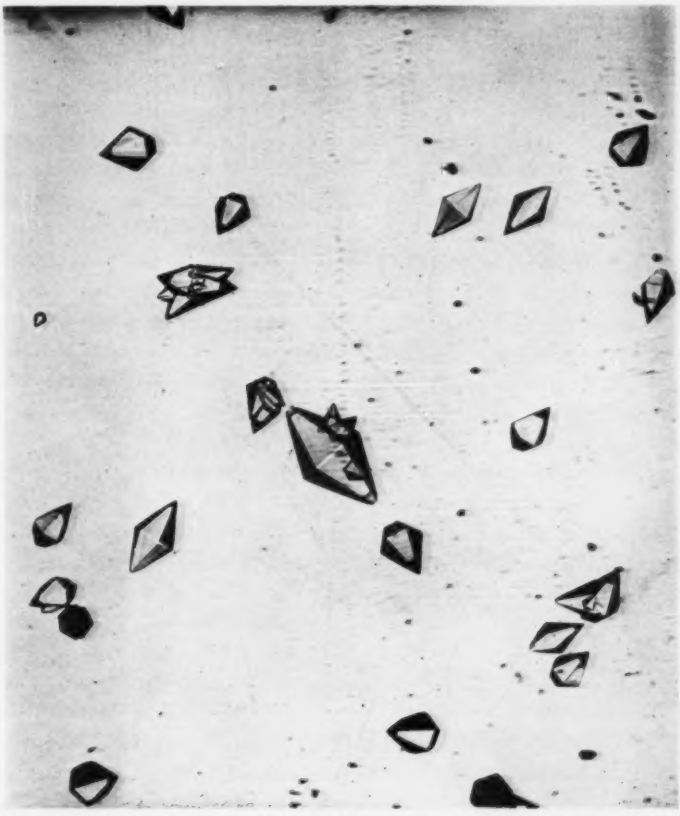


FIG. 12. ACETYL PEPSIN. 60 PER CENT ACTIVE. MAGNIFICATION × 28 (HERRIOTT)

time regains its original activity. Those acetyl groups, which decrease the activity and are removed by standing in acid, are attached to the hydroxyl group of tyrosine. Substitution of iodine for the hydroxyl

groups of tyrosine also decreased the activity. These results show that the free amino groups of pepsin are not necessary for the activity, but that the phenolic groups of tyrosine are essential to the enzymatic activity.

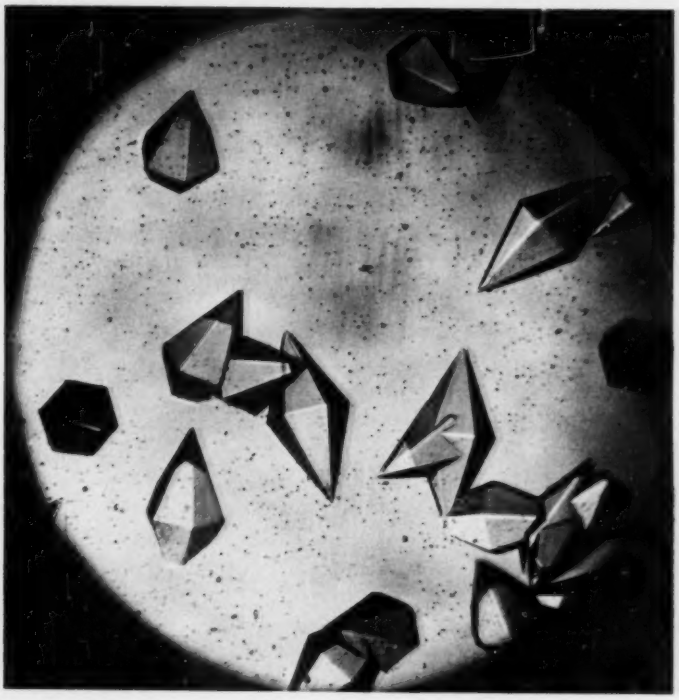


FIG. 13. ACETYL PEPSIN. 100 PER CENT ACTIVE. MAGNIFICATION × 34 (HERRIOTT)

FORMATION OF ENZYMES FROM THEIR INACTIVE PRECURSORS

Further knowledge concerning the chemical structure responsible for the enzymatic activity of the molecule has been obtained by a study of the formation of the active enzymes from their inactive precursors.

Chymo-trypsin from chymo-trypsinogen. Heidenhain and Kühne showed that freshly secreted pancreatic juice was inactive but becomes

active when mixed with the secretion of the small intestine. It was assumed, therefore, that the enzymes were present in the pancreas in an inactive form. The formation of the active enzymes from inactive pancreatic juice or from inactive extracts of pancreas has been extensively studied, but the literature is surprisingly confused and contradictory. The course of the reaction has been described as catalytic, stoichiometric and autocatalytic. These contradictory results were obtained with crude preparations and under the conditions of the various experiments are probably all correct. During the course of the work on the isolation of the inactive form (chymo-trypsinogen) of chymo-trypsin and of the inactive form (trypsinogen) of trypsin we also obtained a number of apparently inexplicable, contradictory results. These varying results were eventually found to be caused by the presence in amorphous chymo-trypsinogen preparations of more or less trypsinogen and of a substance which inhibits trypsin. Small amounts of these impurities influence the activation very markedly and until the protein was crystallized the activation experiments could not be repeated with certainty. When the chymo-trypsinogen has been recrystallized several times it is freed from inhibitor and trypsinogen, and its activation by trypsin becomes a perfectly definite and reproducible catalytic reaction. The rate is proportional to the trypsin concentration and also to the chymo-trypsinogen concentration. The rate of activation by trypsin is affected by the acidity of the solution in the same way as is the rate of digestion of other proteins by trypsin so that the reaction appears to be analogous to ordinary protein hydrolysis. There is one striking difference, however, in that, so far as we have been able to determine, nothing is split from the chymo-trypsinogen molecule, although there is an increase of 5 amino groups per mol. The formation of the active form from the inactive form of chymo-trypsin, therefore, may be due to the opening of a peptide ring. The isoelectric point is shifted from about pH 5.0 to about pH 5.4 and there is a slight decrease in optical rotation, but no other chemical differences have been detected so far. The elementary analysis, the tyrosine plus tryptophane content, the diffusion coefficient and molecular weight of the enzyme and of its precursor are the same within the experimental error. The x-ray defraction patterns of the two substances, according to Wyckoff, are quite different

and show no more resemblance than would be expected between any two different proteins. Ten Broeck has shown that the two proteins may be distinguished by means of the anaphylactic test.

In addition to the activation by trypsin, very slow activation occurs in slightly acid solution. The activation is not accelerated by pepsin, chymo-trypsin itself, enterokinase or trypsinogen.

Trypsin from trypsinogen. As in the case of chymo-trypsinogen the activation of trypsinogen changes markedly after crystallization. Amorphous preparations are stable in solution and can only be activated by the addition of kinase (an extract of the small intestine), by large quantities of trypsin or by the addition of concentrated magnesium or ammonium sulfate solutions. After crystallization, however, the protein becomes transformed very rapidly into the active enzyme as soon as it is dissolved in neutral solution. This change in behavior is due to the fact that amorphous preparations contain traces of a substance which inhibits trypsin and so prevents the autocatalytic reaction. As soon as this inhibitor is removed by crystallization the most minute trace of trypsin starts the activation reaction, which then proceeds autocatalytically. This reaction agrees quantitatively with the theory for a simple autocatalytic reaction. The velocity is affected by acidity and temperature in the same way as is the activation of chymo-trypsinogen or hydrolysis of other proteins by trypsin. At pH 5.0 and 8°C. the value of the constant is 14.6, *i.e.*, with unit concentration of trypsinogen the active trypsin would increase 14 times per hour. This reaction evidently has a formal resemblance to bacterial growth curves. The addition of trypsin inhibitor to such solutions causes the production of a long lag period, and the curves obtained under these conditions are very similar to the usual bacterial growth curves. As in the case of chymo-trypsin there is no evidence for the splitting off of any part of the molecule during this activation reaction nor is there any marked change in chemical composition so that the change from the inactive protein to the enzyme is probably due to some internal rearrangement in the structure of the molecule. However, the work on this question is still in the preliminary stage. The autocatalytic nature of this reaction was correctly described by Vernon but subsequently denied by Bayliss and Starling and others.

Kunitz has recently found that trypsin is formed by trypsinogen at pH 5.0 by a proteolytic enzyme secreted by a mold (Penicillium).

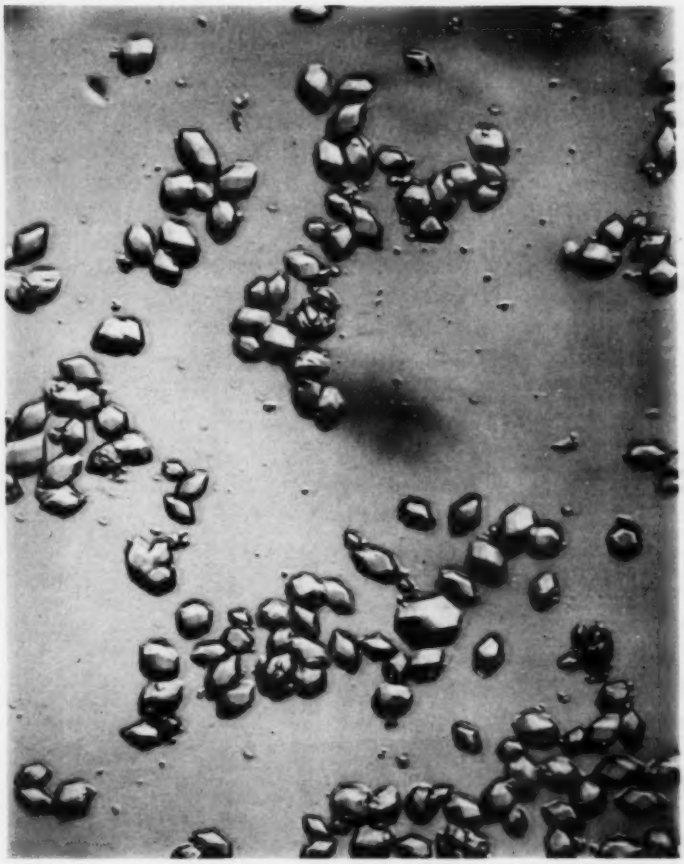


FIG. 14. INHIBITOR-TRYPSIN COMPOUND. MAGNIFICATION × 370 (KUNITZ)

Trypsin is inactive at this pH, so that the activation curve is no longer autocatalytic but simply logarithmic as is the activation of chymotrypsin. Trypsin obtained in this way is identical with that formed

by autocatalytic activation. The mold enzyme, therefore, must attack the molecule at the same place as does trypsin.

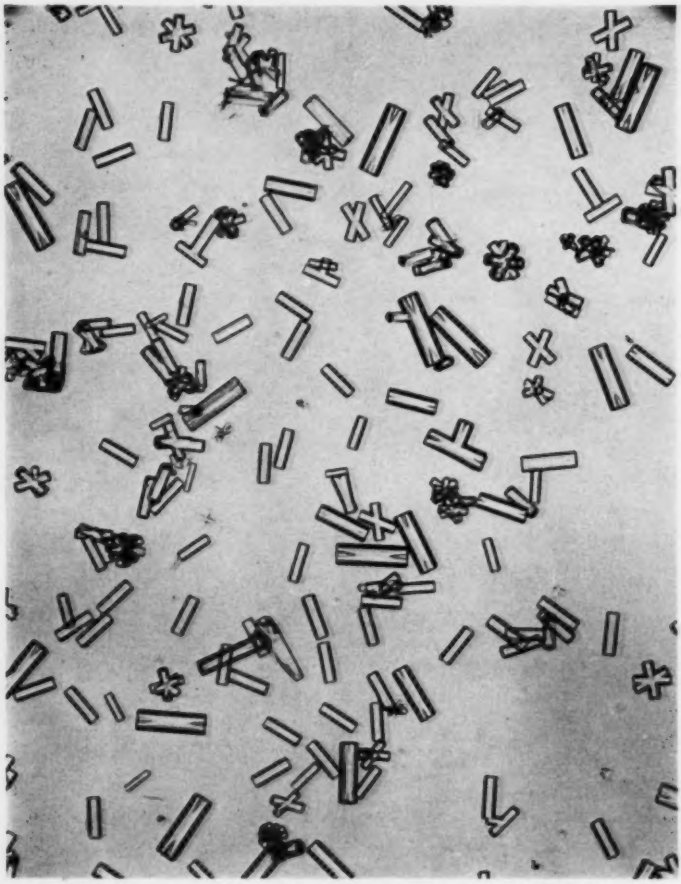


FIG. 15. TRYPSIN INHIBITOR CRYSTALS. MAGNIFICATION × 225 (KUNITZ)

Activation of trypsin by enterokinase. Pancreatic juice as secreted is inactive and becomes active *in vivo* only when mixed with the contents of the small intestine. Schepowalnikow found that this activa-

tion was caused by some substance secreted by the small intestine and which he considered to be an enzyme "enterokinase." Other workers found that the reaction did not proceed like an enzyme reaction but that the quantity of active trypsin formed depended on the quantity of kinase added. They therefore considered the reaction to be stoichiometric and thought that the active trypsin was a compound of the inactive precursor, trypsinogen, and the activator, kinase. The subject has been controversial for many years. Kunitz has reinvestigated the problem with purified materials and has found that the conflicting results are due to the fact that an inert protein, as well as the active enzyme, are formed simultaneously during the reaction. The relative amounts of the two substances obtained depends on the acidity of the solution. In slightly acid solution nearly all the precursor is transformed into the active enzyme and very little inert protein is formed. In neutral or slightly alkaline solution considerable amounts of inert protein are formed.

The entire reaction may be quantitatively predicted on the assumption that the rate of formation of the active enzyme is proportional to the concentration of precursor and of activator, while the rate of formation of the inert protein is proportional to the concentration of trypsin and of the precursor. The relative rates of the two reactions is determined by the acidity.

Pepsin from pepsinogen. Langley showed that pepsin existed in gastric mucosa in a form which differed from the active enzyme in that it was much more resistant to alkali. Herriott has recently succeeded in isolating and crystallizing this substance. It has no proteolytic activity, but in slightly acid solution becomes converted into active pepsin. The reaction at pH 4.65 is autocatalytic and hence is caused by pepsin itself. About 15 per cent of the nitrogen is split off during this reaction. So far as is known, pepsin attacks only peptide linkages so that there is reason to believe that the rupture of one or more peptide links in the precursor leads to the formation of the active enzyme. If swine pepsinogen is activated by chicken pepsin, swine pepsin is formed. The structure responsible for the species specificity of the enzyme therefore is present in the precursor.

Formation of enzymes and bacteriophage by microorganisms. The foregoing sections have described the formation of enzymes from their

precursors. This reaction consists in the transformation of an inert protein into an active enzyme. These reactions in some cases are autocatalytic and therefore present a formal analogy with the growth of cells and the increase in viruses. There is an essential difference between the growth of cells and the formation of enzymes in that cells can use energy to synthesize themselves from simpler compounds whereas, so far as is known, enzymes cannot. It is not yet known whether phage or other viruses possess this power of synthesis. Until this knowledge is obtained it does not seem possible to decide as to which of the two analogies has a real physical basis and which is merely formal. In the meantime it appears to the writer that the much more simple analogy with enzyme formation has predicted the facts better than the analogy with cell growth. Thus, some viruses and phages, at least, are proteins and may be isolated by the methods of enzyme chemistry; they may be inactivated and reactivated; they have no measurable respiration. Pepsin and trypsin are immunologically distinct from their precursors, just as phage and virus are immunologically distinct from their host cells.

The curves for the rate of formation of phage and of enzymes by cells or from their precursors are similar and more or less autocatalytic in character.

It is evident from the foregoing that a formal analogy exists between the production of phage and the formation of enzymes. If this analogy has any real physical significance the simultaneous formation of phage and of an extracellular enzyme in the same culture should follow very similar curves. In order to test this prediction the rate of formation of phage and of a gelatinase by lysogenic and "sensitive" strains of *B. megatherium* has been determined under a variety of conditions. In general the curves for the increase of phage and of gelatinase are very similar. There is a rapid increase in phage and enzyme concentration during the growth stage of the bacteria. The increase in all these quantities is logarithmic (autocatalytic); *i.e.*, the amount formed is proportional to the amount already present. The concentration of phage and gelatinase reaches a maximum and then decreases. The decrease may or may not be accompanied by a decrease in the number of cells depending upon the conditions and strain of bacteria used. The production of phage may be separated from that of gelatinase by

varying the calcium concentration. In the presence of high calcium concentration little phage is produced while the gelatinase increases to a measurable extent, but less than it does in low calcium concentration. In the absence of calcium no phage is produced and the production of gelatinase is decreased.

Merrill and Clark have found that the production of enzymes by certain bacteria also requires calcium.

THE EFFECT OF INSULIN ON THE GLYCOGEN CONTENT OF ISOLATED MUSCLES[1, 2]

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There have been numerous attempts to demonstrate an *in vitro* effect of insulin on muscular tissue. Soon after its discovery, Ahlgren (1) reported that the addition of this hormone to washed muscles in the presence of glucose, reduced the decolorization time of methylene blue under anerobic conditions. These findings were confirmed by Blix (7), Markowitz (28) and von Euler (16), although von Euler was able to obtain this effect only when cozymase was added to the mixture. Narita (30), using higher concentrations of insulin, could not repeat Ahlgren's finding. Ahlgren observed also that high concentrations had no effect, and positive results were found only when very small amounts of insulin were used. The reaction was highly specific, for glucose (Ahlgren (1)) and glycerol (von Euler (16)) were the only two of a large number of substances which gave a positive reaction.

Another approach to this problem is that of measuring the oxygen consumption of isolated muscles with and without insulin in the medium. Ahlgren (2) and Brahme (8) described an increase in oxygen consumption on the addition of insulin to minced muscle. Büchner and Grafe (11) obtained a similar result on muscle slices. Takane (35) observed a 20 per cent increase in oxygen consumption when insulin was present in serum surrounding the rat's diaphragm. Paasch and Reinwein (31) and Rothschild (32) could not confirm these earlier observations. Von Euler (16) obtained positive results when the

[1] This work was made possible by a grant from the Rockefeller Fluid Research Fund.

[2] Preliminary reports of this work were presented before the American Physiological Society in April, 1939, at Toronto, Canada, and in March, 1940, at New Orleans, La.

6770

oxygen tension was reduced to 10 per cent or less. The most recent work on this subejct is that of Shorr and Barker (33) who used both tissue slices and minced muscle. These authors reported that insulin had no stimulative effect on respiration of muscle, and, in some cases, it even depressed respiration. Krebs and Eggleston (22) demonstrated a remarkable increase in oxygen uptake of minced washed muscle of pig ons when insulin, citrate and boiled muscle extract were added. These authors, dismissing all other effects of insulin as side reactions, claim that their experiments prove that insulin aids in the oxidation of carbohydrate in the peripheral tissues. Unfortunately for their general theory, similar results could not be obtained by Shorr and Barker (33) when minced muscle from chickens, cats, dogs or rabbits was used. These authors, however, did obtain a slight increase in oxygen consumption when pigeon's breast-muscle was used. Stadie, Zapp and Lukens (34) reported results similar to those of Shorr and Barker. Pigeon's muscle must have a highly specialized mechanism for its oxidative process.

The action of insulin in phosphorylating systems has also been investigated. Brugsch and Horsters (10) described an accelerated disappearance of inorganic phosphate in the presence of insulin. These results could not be confirmed by von Euler (15). Lehmann (23) and Gill and Lehmann (18) demonstrated recently that the formation of glucose-6-phosphate from glucose-1-phosphate or from glycogen was inhibited by insulin. These authors ascribed this effect to an interaction of the protein molecule with magnesium ions. This reaction was not specific, for other proteins having a similar isoelectric point exerted an inhibiting effect comparable to that of insulin. Cori (13) stated that he could confirm Lehmann's results with commercial insulin but not when a zinc free water soluble insulin powder was used in similar experiments. Gill and Lehmann (18) demonstrated, however, that various samples of insulin containing both large and small amounts of zinc, possessed the same inhibitory effect.

In addition to studying the effect of insulin on the oxygen consumption of isolated tissues, Takane (35) determined the carbohydrate balance in the tissues. He reported that insulin accelerated to a slight degree the carbohydrate utilization by the rat's diaphragm.

These various experiments have given no definite evidence for the

site of the action of insulin in muscle. The methylene blue experiments of Ahlgren were positive only when very small amounts of impure insulin were used and the difference in reduction time was only 20 per cent. The recent work of Shorr and Barker ruled out the possibility of any increase in respiratory metabolism of isolated muscle due to insulin. Krebs' experiments were valid only when applied to minced pigeon's pectoral muscle. Lehmann's inhibitory effect was not specific for insulin. In view of these negative findings on oxidative mechanisms, it was decided to study the effect of insulin on a synthetic process, that of glycogen formation from glucose with the hope, if insulin did accelerate this process, that this preparation might be used for future studies on the site of insulin action in muscle.

METHODS

Frogs' sartorii and rats' diaphragms were used in these experiments. When frogs' muscles were employed corresponding sartorii were dissected out, washed in Ringer's solution and placed in separate Warburg vessels. In the majority of the experiments, three muscles were used in each vessel in order to obtain adequate amounts of tissue for glycogen determinations. 5.0 ml. of Ringer's solution, made according to the directions of Barkan, Broemser and Hahn (4), were placed in each vessel. This solution contained 0.1 per cent glucose. 2.5 per cent carbon dioxide in 97.5 per cent oxygen was passed through each vessel for ten minutes. The temperature of the water bath was kept constant at 15.9 $\pm$ 0.1 degree Centigrade. Amorphous insulin which assayed 22 units to the milligram was used in all of the frog-muscle experiments. Control experiments were made in which the Ringer's solution did not contain insulin. The vessels were shaken at the rate of forty times per minute.

In the experiments on rat's diaphragm, the rat was killed by a blow on the head. The diaphragm was excised quickly and placed in cooled Ringer's solution. It was divided into two parts by cutting along the central ligament and discarding the posterior muscle portion. Each lateral half was washed again in Ringer's solution, dried quickly on filter paper and placed in a separate Warburg vessel. Each vessel contained 2 ml. of Ringer's solution. This solution was made according to the directions given by Gey and Gey (17) with the exception that it did not contain the magnesium salts and did contain 0.2 per cent glucose in the majority of the experiments. In the others, 0.1 per cent glucose was used. The pH of the solution was adjusted to approximately 7.3 by the addition of phosphate. The solutions containing bicarbonate were equilibrated in the vessels with 5.0 per cent carbon dioxide and 95 per cent oxygen. In the majority of the experiments sodium bicarbonate was absent from the solutions. In these, oxygen was passed through the vessels for five to ten minutes. The temperature of the

bath when the rat's diaphragm was used was kept at 37.2 ± 0.1 degree Centigrade. In a small series of experiments, oxygen determinations were made. In these, the vessels were shaken at the rate of 120 times per minute. In the experiments in which only glycogen was determined the vessels were shaken at the rate of 80 times per minute.

In both the frog and the rat experiments, the muscles were removed from the vessels, dried quickly on filter paper and placed in potassium hydroxide. The glycogen was determined by the method of Good, Kramer and Somogyi (19). The results were reported in terms of per cent glucose. Amorphous zinc insulin which contained 22 units per mgm., and a commercial solution of crystalline zinc insulin (Lilly) which assayed 40 units per ml. were used in the rat diaphragm experiments. The solution of commercial crystalline zinc insulin contained a very small amount of glycerol and phenol.

The rat diaphragm was selected for these experiments because of its thinness and the ease of dissection without trauma. Two determinations of thickness of portions of the same diaphragm gave 0.38 mm. and 0.42 mm. Histological sections were made of two strips of diaphragm, one after fifteen minutes of shaking in Ringer's solution and the other after three hours. The tissues were fixed in Bouin's solution and stained with haematoxylin and eosin. Examination of these sections showed, in both cases, that there were cells in the center of the section which did not take the stain, and which under high-power magnification showed signs of membrane rupture. Therefore, these experiments must be interpreted in the same manner as any other tissue-slice experiment: there is the possibility of cellular damage modifying the results. Since previous experiments on frogs' sartorii and rats' diaphragm had demonstrated that the respiratory metabolism remained constant over periods of six and three hours respectively, these periods were adopted for the experiments. The cellular damage could not be very extensive in these time intervals since the rate of oxygen consumption was approximately the same at the end as at the beginning of the experiments.

RESULTS

The results of the glycogen determinations are given in Figs. 1–4 inclusive. A point was obtained, in the control series, by subtracting the value of glycogen in terms of per cent of one muscle from the value of glycogen obtained on the corresponding strip of muscle, neither muscle having been in contact with insulin. In the series with insulin, a point was obtained by subtracting the value of glycogen of the control muscle from the value obtained from the corresponding muscle which had been in contact with insulin. For example, if the control muscle contained 0.30 per cent glycogen and the corresponding muscle with insulin 0.50 per cent, the difference, + 0.20 per cent, would be plotted on the graph as one point.

The results of the determinations on the frog muscles are given in Fig. 1. The general average of the differences of glycogen content between the two sets of muscles in the control series was −0.01 per cent. When insulin was added, the average figure was not changed to a significant degree; the value was +0.02 per cent glycogen. Since the general average difference in the series with insulin was of the same order of magnitude as in the control series, it was concluded that in-

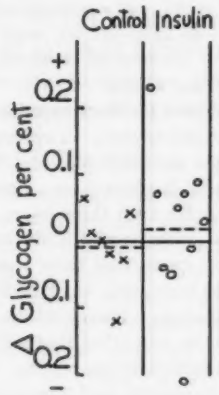

FIG. 1. THE EFFECT OF INSULIN UPON THE GLYCOGEN CONTENT OF FROGS' SARTORII

In the control column the differences are given for the glycogen content in terms of per cent of two sets of muscles shaken for periods of 5 to 6 hours without insulin in the medium. In the insulin column, the differences are given for the glycogen content of two sets of muscles shaken for periods of 5 to 6 hours with insulin in concentrations ranging from 0.1 to 2.0 mgm. per cent in the medium in one vessel. The dotted lines represent the average of each group. See text for method of calculation of differences.

sulin under the conditions of these experiments had no influence on the glycogen content of frogs' sartorii.

Positive results were obtained, however, when insulin was added to the medium in the rat diaphragm experiments (Fig. 2). In the control series, a general average difference of −0.01 per cent was obtained with deviations from +0.14 to −0.14. In the experiments in which insulin was added to one muscle, the average of the deviation is +0.25

per cent with a maximum of +0.47 and a minimum of −0.12 per cent. The average value of the per cent glycogen in the control group was 0.44 and in the insulin group was 0.69, an increase of 57 per cent over the control value. There can be no doubt that the increase in glyco-

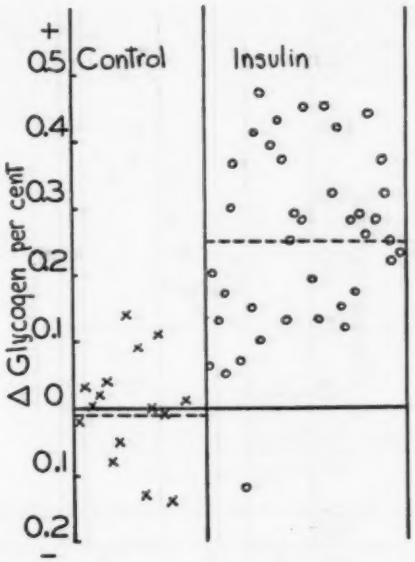

FIG. 2. THE EFFECT OF INSULIN UPON THE GLYCOGEN CONTENT OF RATS' DIAPHRAGM

In the control column the differences are given for the glycogen content in terms of per cent of two sets of muscles shaken for periods of 3 hours without insulin in the medium. In the insulin column the differences are given for the glycogen content of two sets of muscles shaken for 3 hours with insulin in concentrations from 0.03 to 24.4 mgm. per cent in the medium in one vessel. The dotted lines represent the average of each group.

gen represents a definite change in the muscle exposed to the action of insulin.

In another series of experiments the initial value of the glycogen content was compared with the value after three hours of shaking in order to determine whether the insulin was inhibiting the breakdown

of glycogen or aiding in a synthetic production of glycogen (Fig. 3). Determinations were also made on corresponding muscles after fifteen minutes of shaking. The average deviation was +0.02 per cent glycogen, demonstrating that the two muscle strips had comparable

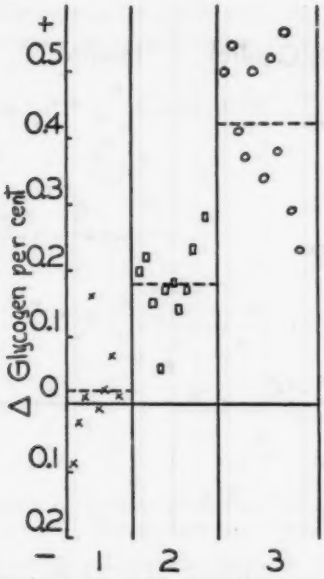

FIG. 3. EFFECT OF INSULIN UPON GLYCOGEN CONTENT OF RATS' DIAPHRAGM

Column 1: Differences in glycogen content in terms of per cent between corresponding sets of diaphragmatic muscles both shaken for fifteen minutes without insulin. Column 2: Similar differences between corresponding sets of muscles one of which was shaken for fifteen minutes and the other for 3 hours. No insulin in either medium. Column 3: Similar differences between corresponding sets of muscles, one of which was shaken for fifteen minutes and the other for three hours. Insulin in concentrations from 3.6 to 6.7 mgm. per cent was present in the medium in the vessel shaken for three hours.

glycogen contents at the start of the experiment. The maximal was +0.16 and the minimal was −0.09. In another series, when the initial value was compared to the value after three hours of shaking without insulin, the average deviation was +0.18 with a maximum

value of +0.28 and a minimum of +0.05. In the third series, when insulin was added to one muscle, the deviation between the initial and final values increased to +0.42 with a maximum value of +0.56 and a minimum of +0.23. Since the average of this series with in-

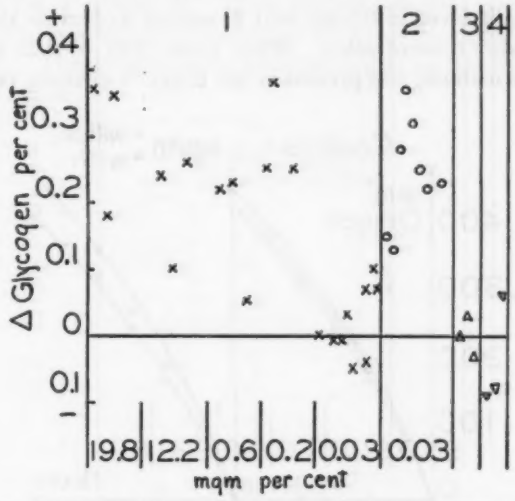

FIG. 4. CONTROL EXPERIMENTS WITH DIAPHRAGM FROM RATS

In Column 1 results of experiments with phenylisocyanate-insulin. The differences are given for the glycogen content in terms of per cent for two sets of muscles shaken for three hours with phenylisocyanate-insulin in concentrations ranging from 19.8 to 0.03 mgm. per cent in the medium of one vessel. In column two, the results of similar experiments in which amorphous insulin in concentrations of 0.03 mgm. per cent was used. In column three, the results of three experiments in which 0.60 mgm. per cent ovalbumin was present in one vessel. In column four, results of three experiments in which insulin was present in one vessel and glucose was absent from both vessels.

sulin was greater than the one without, these experiments demonstrate that insulin was aiding in a synthetic production of glycogen.

Several methods were used for controlling these experiments. In order to use a protein molecule comparable to the insulin molecule but not having the same physiological action, numerous inactivated preparations of insulin were considered. Dr. Jensen suggested that

phenylisocyanate-insulin had the best properties of an inactivated insulin for these experiments and he supplied a preparation of this material made from amorphous insulin. The inactivation of insulin by phenylisocyanate has been studied by Hopkins and Wormall (20) and by Jensen and Evans (21). The material used in these experiments was dissolved in 0.5 ml. 0.01 N sodium hydroxide and diluted to the needed concentration. When from 19.82 to 0.22 mgm. per cent of this material was present in the Ringer's solution, positive re-

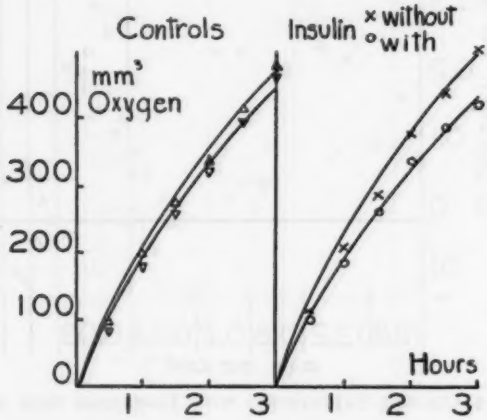

FIG. 5. EFFECT OF INSULIN UPON THE OXYGEN CONSUMPTION OF RATS' DIAPHRAGM

Total oxygen consumption of two sections of diaphragm plotted against time in hours. Weight of muscles in control experiment: 0.161 and 0.151 grams. 6.7 mgm. per cent zinc insulinate used in insulin experiment. Weight of muscles: without insulin, 0.179 grams; with insulin, 0.183 grams.

sults were obtained comparable to those found with active insulin (Fig. 4). However, phenylisocyanate-insulin retains about five per cent of its original physiological activity. For an adequate control, therefore, a concentration must be reached at which this small residual activity would have no effect while active insulin, at the same concentration, would give a physiological response. This was achieved at 0.03 mgm. per cent (Fig. 4). These experiments demonstrate that insulin is active in these preparations in concentrations somewhere between 0.03 mgm. per cent and 0.0015 mgm. per cent. When the

higher figure is converted into units present in 2 ml. of solution a value of 0.013 units of insulin is obtained demonstrating that this amount has physiological activity *in vitro*. Three experiments with recrystallized ovalbumin showed that this protein had no effect on the muscle glycogen (Fig. 4, column 3). Three experiments were made in which insulin was added to Ringer's solution not containing glucose (Fig. 4, column 4). These experiments gave no change in the glycogen content of the muscle.

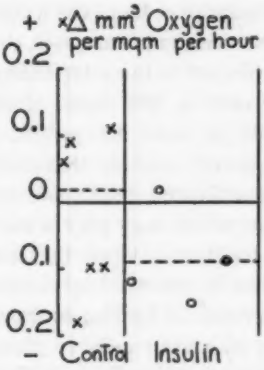

FIG. 6. EXPERIMENTS SHOWING EFFECT OF INSULIN ON OXYGEN CONSUMPTION OF RATS' DIAPHRAGM

Differences in oxygen consumption in terms of mgm. wet weight of tissue per hour. In the control column differences are given for the oxygen consumption of two corresponding strips of muscle without insulin in the medium shaken for three hours. In the insulin column the differences are given for two sets of muscles one of which was in a medium containing 6.7 mgm. per cent of insulin. Average oxygen consumption of all control experiments was 0.93 mm.^{3} O_2 per mgm. wet weight of tissue per hour; with insulin it was 0.84 mm.^{3}

The experiments on oxygen consumption of rats' diaphragm showed no marked differences between those with and without insulin. (Figs. 5 and 6.)

DISCUSSION OF RESULTS

The negative results obtained with the frog sartorii probably were due to the slow velocity of chemical processes in that muscle. The positive experiments with the rat's diaphragm were interpreted as an

accleration of glycogen formation from glucose in the isolated muscle. This conclusion was based on (a) the comparison of the final values of glycogen content in the muscle after three hours of shaking, (b) the increase in glycogen over the initial level, when insulin was present, (c) the failure of an inactivated insulin in low concentrations to give positive results, (d) the failure of another protein, ovalbumin, to give positive results, and (e) the negative results obtained when glucose was not present in the medium. The results were reported in terms of per cent which involves two variables, the glycogen determinations and the weights of the muscles. Since the weights of the muscles were reported in terms of wet weight a change in the water content of the muscle may have contributed to the total change. In order to get an increment of 0.25 per cent in 100 mgm. of tissue, when the initial glycogen content is 0.44 per cent, the weight of the tissue must decrease 35 per cent. It is very unlikely that insulin would produce this effect especially when inactivated insulin and ovalbumin give negative results. Another factor which may play a role is the change of permeability of the cell to glucose when the insulin is present. The literature on this subject is reviewed by Ammon (3) and additional experiments have been reported by him to show that insulin does not affect the permeability of muscle cells to glucose. Therefore, these experiments demonstrate that insulin is aiding in the formation of glycogen in the isolated muscle.

Numerous other lines of evidence have demonstrated a similar effect. Best and his associates (6) found an increase in the storage of glycogen when insulin was present in the eviscerated spinal animal, Long and Horsfall (24) in the decapitated eviscerated cat, and Major and Mann (27) in the depancreatized, dehepatized dog. Bridge (9) has demonstrated a storage in muscle proportional to the amount of insulin given to a normal animal receiving glucose over a long period of time. Insulin is aiding, therefore, in accelerating a reaction which is not completely lost by the removal of this hormone. Major and Mann (27) found that a diabetic animal could store glycogen if glucose was given, but not to the same extent as the normal. Barker and Sweet (5) demonstrated that considerable storage occurred in the diabetic animal if high concentrations of blood glucose were maintained. Additional evidence has come from a study of the reformation of

glycogen following stimulation of muscles in a diabetic. All workers agree that the resynthesis is impaired, although they disagree as to the extent of the impairment (Milroy (29), Debois (14), Lukens (25), Cleghorn and Peterson (12), McKay (26)). These findings demonstrate also that insulin aids in the conversion of glucose to glycogen in muscle.

SUMMARY OF RESULTS

The effect of insulin upon muscle glycogen was investigated by determining the glycogen content of isolated muscles which had been suspended for a suitable time in glucose-containing Ringer's solution with and without the addition of insulin. The glycogen content of frogs' sartorii was not affected by the presence of insulin in the surrounding medium. On the other hand, rat's diaphragm showed an appreciable increase in glycogen when insulin was present. When this change was compared to the initial value, a definite synthetic production of glycogen was found. If the surrounding medium contained no glucose, or when ovalbumin or small amounts of inactivated insulin were substituted for active insulin, the increase in muscle glycogen did not take place. Insulin did not increase the oxygen-uptake of rat's diaphragm. These results are discussed in relation to previous work upon normal and diabetic animals.

The author wishes to thank Dr. H. Jensen for his generous gift of amorphous insulin and phenylisocyanate insulin, Dr. L. Hellerman for supplying the recrystallized ovalbumin and Miss E. Lambert for making the histological preparations.

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STUDIES ON THE CELLULAR RESPONSE IN THE EARLY STAGES OF THE TUBERCULIN REACTION

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It is well established that when a foreign protein is injected into the skin of an animal hypersensitive to that protein one of two reactions may occur. The response may appear promptly and manifest itself by erythema and oedema or its appearance may be delayed and then take several hours to reach its full intensity. The first response has been termed the anaphylactic type of reaction, the Arthus phenomenon being an exaggerated form of this. Since antibody is demonstrable, passive transfer is easily accomplished. The second type has been called the bacterial or tuberculin type of skin hypersensitivity. Antibody cannot be demonstrated by ordinary methods and passive transfer has not been satisfactorily accomplished. Another fundamental difference between these two types of hypersensitivity has been elicited by the behavior of cells in tissue culture. Specific foreign protein added to cells from an animal displaying the anaphylactic type of skin response is found to be perfectly innocuous to those cells (1). On the other hand, Rich and Lewis (2) and others (3, 4) have shown that cells from tuberculous animals are killed by tuberculoprotein, while normal cells are not damaged.

In 1932, after studying the histological changes taking place in these two types of reaction in the guinea pig, Dienes and Mallory (5) reported that while in the early stages of the anaphylactic skin reaction there was an intense polymorphonuclear leucocytic response, the tuberculin reaction was characterized from the beginning by a predominance of mononuclear cells. Thus they felt justified in adding this differential point to the other differences which separate these two types of hypersensitivity.

Dienes and Mallory (6) found that "the mononuclear type of reaction develops in purest form in slight reactions and in the early period after infection." They (5) stated that "the mononuclear preponderance is greatest in the early stages, up to 6 hours, and again in the late stages from 48 hours on." They considered that a polymorphonuclear reaction was a response to the necrosis of epithelium.

Similar histological studies on tuberculin reactions were reported by Laporte (7) who stated at one place in his summary that in slight reactions "the monocytes predominate in all stages of the reaction." However, after a careful examination of Laporte's protocols we do not believe that such a conclusion is justified since he does not specifically describe the appearance of slight reactions until the 20th hour. Laporte at another place described the predominance of polymorphonuclear cells in the dermis in the early stages, and he further states that in the first hours, there is an effluxion of polymorphonuclears while the macrophage reaction occurs later.

Feldman and Fitch (8) studied the histological appearance of tuberculin reactions in calves and found that polymorphonuclear cells predominated in the early stages. In discussing the observations of these authors, Long (9) stated that he and his co-workers had observed "a polymorphonuclear reaction beginning in a few hours and lasting up to two days."

In the initial stages of any inflammatory reaction a predominately mononuclear response is unique. Because of the importance of the observation of Dienes and Mallory (5) and since it has not been satisfactorily confirmed, we felt that it was worth while to repeat their experiments, using small amounts of tuberculoprotein or animals of low sensitivity as emphasized by Dienes (6).

MATERIALS AND METHODS

Adult white male guinea pigs were used. They were rendered hypersensitive by the inoculation of a heavy suspension of human type tubercle bacilli into the groin. After the appropriate interval (8–35 days) the skin on each side was plucked and shaved and the next day the animals were injected intracutaneously at different sites with 0.1 cc. of the desired dilution of Old Tuberculin and 0.1 cc. of the medium control solution. The skin sites were removed at varying intervals, cut into several blocks, care being taken to include the center of skin

lesion, fixed in Zenker-formol and stained with haematoxylin and eosin. At the site of maximum reaction of each intracutaneous test 200 cells were counted differentially with the oil immersion lense of the microscope. The Old Tuberculin used was made by growing a virulent human strain of tubercle bacilli on Proskauer and Beck's medium for 8 weeks. The organisms were partially removed by filtration through paper; the filtrate was then passed through a Berkefeld N candle and finally evaporated to one-tenth the original volume on the water bath. In Series IV Seibert's purified protein derivative was used (P.P.D., Parke, Davis and Company). Control injections with concentrated medium in the appropriate dilutions were also employed. Injections of Old Tuberculin into normal animals were also carried out. The tuberculoprotein used by Dienes and Mallory was "a filtrate of virulent cultures on Long's synthetic medium, either unconcentrated or concentrated by $(NH_4)_2SO_4$ precipitation or ultrafiltration. In certain guinea pigs even 0.0002 cc. of a 10 × concentrated filtrate was too much as it produced necrosis. In other animals 0.01 cc. was necessary to produce a noticeable reaction" (6). The amounts of tuberculoprotein used in the present experiments are similar to those used by Dienes and Mallory. Moreover the intensity of the reactions conforms to that described in their paper.

RESULTS

Series I

This consisted of four tuberculin sensitive animals which had been infected 5 weeks before. They were injected intracutaneously with 0.1 mg. Old Tuberculin and the same dilution of control medium. At the site of injection very little was noted grossly. After half an hour the initial bleb had disappeared. There was slight erythema and a little swelling by the 5th to 6th hours. No necrosis was noted. The sites were examined 2 to 20 hours after injection. In Table I are shown the time interval in hours between the skin testing and removal, the substance used in the test and the percentage of polymorphonuclear leucocytes, mononuclear cells, and connective tissue cells in a total of 200 cells counted.

From this table it is obvious that in the early hours of the reaction (2nd to 8th) the cellular reaction is predominantly a polymorphonuclear one and in no case were the mononuclear cells in excess as noted by Dienes and Mallory (5). In the later hours the percentage of polymorphonuclear cells diminishes somewhat but even by the 20th hour the cellular response still shows a preponderance of granulocytes. On the whole there is little difference between the Old Tuberculin and control medium reactions. It must be noted, however

TABLE I

ANIMAL NUMBER	TIME IN HOURS AFTER SKIN TEST	MATERIAL INJECTED*	PERCENTAGE OF CELLS AT SITE OF REACTION†			ANIMAL NUMBER	TIME IN HOURS AFTER SKIN TEST	MATERIAL INJECTED*	PERCENTAGE OF CELLS AT SITE OF REACTION†		
			P.M.N.	Mon.	C.T.				P.M.N.	Mon.	C.T.
29	2	0.1 mg. O.T.	76	12	12	27	10	0.1 mg. O.T.	67	22	11
	2	0.1 mg. C.	75	14	11		10	0.1 mg. C.	74	18	8
	4	0.1 mg. O.T.	72	14	14		12	0.1 mg. O.T.	61	29	10
	4	0.1 mg. C.	80	7	13		12	0.1 mg. C.	58	34	8
28	6	0.1 mg. O.T.	70	21	9	26	16	0.1 mg. O.T.	50	38	12
	6	0.1 mg. C.	67	25	8		16	0.1 mg. C.	56	31	13
	8	0.1 mg. O.T.	65	25	10		20	0.1 mg. O.T.	53	39	8
	8	0.1 mg. C.	63	28	9		20	0.1 mg. C.	38	53	9

* O.T., old tuberculin; C., medium control.
† P.M.N., polymorphonuclear; Mon., mononuclears; C.T., connective tissue cells.

TABLE II

ANIMAL NUMBER‡	TIME IN HOURS AFTER SKIN TEST	MATERIAL INJECTED*	PERCENTAGE OF CELLS AT SITE OF REACTION†			ANIMAL NUMBER‡	TIME IN HOURS AFTER SKIN TEST	MATERIAL INJECTED*	PERCENTAGE OF CELLS AT SITE OF REACTION†		
			P.M.N.	Mon.	C.T.				P.M.N.	Mon.	C.T.
45 TB.	4	3 mg. O.T.	81	9	10	45 TB.	6	3 mg. O.T.	72	17	11
45 TB.	4	3 mg. C.	71	20	9	45 TB.	6	3 mg. C.	72	19	9
48 N.	4	3 mg. O.T.	81	12	7	48 N.	6	3 mg. O.T.	75	16	9
48 N.	4	3 mg. C.	75	12	9	48 N.	6	3 mg. C.	67	22	11
44 TB.	4	1 mg. O.T.	73	17	10	44 TB.	6	1 mg. O.T.	63	28	9
44 TB.	4	1 mg. C.	57	30	13	44 TB.	6	1 mg. C.	66	24	10
47 N.	4	1 mg. O.T.	79	13	8	47 N.	6	1 mg. O.T.	61	27	12
47 N.	4	1 mg. C.	79	12	9	47 N.	6	1 mg. C.	63	28	9

* O.T., old tuberculin; C., medium control.
† P.M.N., polymorphonuclears; Mon., mononuclears; C.T., connective tissue cells.
‡ TB., tuberculin sensitive; N., normal.

that the latter were less intense. Necrosis of the overlying epithelium was not noted in the early stages.

Series II

This comprised 4 guinea pigs, 2 of which were tuberculin hypersensitive, having been infected 4 weeks before, while 2 were normal. They were injected intracutaneously with 1.0 and 3.0 mg. Old Tuberculin together with the appropriate dilution of control medium. Skin was removed at 4 and 6 hours after the test.

In Table II are shown the pertinent data and results of the microscopical study of the cellular reaction. It will be seen that at 4 and 6 hours after the tuberculo-protein was injected, the cellular reaction in both tuberculous and normal animals was preponderantly a polymorphonuclear one, the proportions being about the same whether the animals were hypersensitive or not. No necrosis of the overlying epithelium was noted save at the point where the needle had entered the epidermis.

Series III

This consisted of 4 guinea pigs all of which were infected with a turbid suspension of virulent tubercle bacilli. In order to have animals of slight sensitivity one guinea pig was skin-tested and killed on each of the 8th, 10th, 12th and 15th days following infection. After each had been injected with 3 mg., 0.3 mg., and 0.03 mg. Old Tuberculin and control solutions, the skin sites were removed from each 4 and 8 hours later.

In Table III are shown the results of microscopic study of these animals. It will be seen that at the 4th and 8th hours, the number of polymorphonuclear cells is much greater than that of the mononuclear variety. In animals receiving 3 mg. O.T. and tested on the 10th, 12th and 15th days, necrosis of the epithelium was noted at 8 hours.

Series IV

This consisted of 2 guinea pigs, one infected 2 weeks and the second infected 4 weeks before skin testing. Both were injected with 0.004 mg. P.P.D. and 0.0025 mg. P.P.D., the sites of injection being removed

TABLE III

ANIMAL NUMBER	DAYS AFTER INFECTION	HOURS AFTER SKIN TEST	MATERIAL INJECTED*	PERCENTAGE OF CELLS AT SITE OF REACTION†			ANIMAL NUMBER	DAYS AFTER INFECTION	HOURS AFTER SKIN TEST	MATERIAL INJECTED*	PERCENTAGE OF CELLS AT SITE OF REACTION†		
				P.M.N.	Mon.	C.T.					P.M.N.	Mon.	C.T.
91	8	4	3.0 mg. O.T.	70	22	8	93	12	4	3.0 mg. O.T.	78	13	9
		4	3.0 mg. C.	68	25	7			4	3.0 mg. C.	81	12	7
		4	0.3 mg. O.T.	67	19	13			4	0.3 mg. O.T.	77	16	7
		4	0.3 mg. C.	71	18	11			4	0.3 mg. C.	75	15	10
		4	0.03 mg. O.T.	65	28	7			4	0.03 mg. O.T.	70	21	9
		4	0.03 mg. C.	69	21	10			4	0.03 mg. C.	74	19	7
		8	3.0 mg. O.T.	80	12	8			8	3.0 mg. O.T.	72	20	8
		8	3.0 mg. C.	78	11	11			8	3.0 mg. C.	76	15	9
		8	0.3 mg. O.T.	69	21	10			8	0.3 mg. O.T.	70	22	8
		8	0.3 mg. C.	72	21	7			8	0.3 mg. C.	74	14	12
		8	0.03 mg. O.T.	68	20	12			8	0.03 mg. O.T.	65	34	11
		8	0.03 mg. C.	65	25	10			8	0.03 mg. C.	71	20	9
92	10	4	3.0 mg. O.T.	82	11	7	94	15	4	3.0 mg. O.T.	78	10	12
		4	3.0 mg. C.	77	12	11			4	3.0 mg. C.	74	17	9
		4	0.3 mg. O.T.	79	13	8			4	0.3 mg. O.T.	68	20	12
		4	0.3 mg. C.	80	10	10			4	0.3 mg. C.	70	22	8
		4	0.03 mg. O.T.	76	15	9			4	0.03 mg. O.T.	79	8	13
		4	0.03 mg. C.	80	12	8			4	0.03 mg. C.	72	18	10
		8	3.0 mg. O.T.	77	12	11			8	3.0 mg. O.T.	80	13	7
		8	3.0 mg. C.	71	20	9			8	3.0 mg. C.	75	15	10
		8	0.3 mg. O.T.	70	17	13			8	0.3 mg. O.T.	70	23	7
		8	0.3 mg. C.	74	17	9			8	0.3 mg. C.	69	22	9
		8	0.03 mg. O.T.	70	16	14			8	0.03 mg. O.T.	74	13	13
		8	0.03 mg. C.	75	15	10			8	0.03 mg. C.	65	24	11

* O.T., old tuberculin; C., medium control.
† P.M.N., polymorphonuclears; Mon., mononuclears; C.T., connective tissue cells.

at 3, 6, and 9 hours after. 0.002 mg. P.P.D. is roughly equivalent to 0.3 mg. Old Tuberculin.

In Table IV are shown the results of the cytological studies on these two animals. The percentage of mononuclear cells never ran above 23% of the total cells counted so that there was as noted in the animals of the above groups a definite preponderance of the polymorphonuclear type. With the larger amount of P.P.D. (0.004 mg.) necrosis of the epidermis was noted microscopically at 6 and 9 hours

TABLE IV

Animal number	Weeks after infection	Time in hours after skin test	Amount P.P.D. injected	Percentage of cells at site of reaction*		
				P.M.N.	Mon.	C.T.
102	2	3	.004 mg.	75	18	7
		6	.004 mg.	78	12	10
		9	.004 mg.	72	19	9
		3	.0025 mg.	80	9	11
		6	.0025 mg.	72	20	8
		9	.0025 mg.	69	18	13
103	4	3	.004 mg.	70	19	11
		6	.004 mg.	78	14	8
		9	.004 mg.	74	19	7
		3	.0025 mg.	78	11	11
		6	.0025 mg.	68	23	9
		9	.0025 mg.	71	19	10

* P.M.N., polymorphonuclears; Mon., mononuclears; C.T., connective tissue cells.

following the test injection. No necrosis occurred following the smaller amount.

SUMMARY

In the experiments described above we have repeated as closely as possible the work of Dienes and Mallory (5), who contended that the tuberculin reaction differs from other forms of acute inflammation in that mononuclear cells predominate at all stages from the beginning of the reaction. Our histological observations on reactions of comparable intensity produced by similar dilutions of tuberculo-protein have shown that in the tuberculous guinea pig at the site of injection of tuberculoprotein the cellular response in the early stages is predominantly a polymorphonuclear one.

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PSEUDO-THROMBOTIC OCCLUSION OF THE COMMON FEMORAL ARTERY BY CYSTIC DISTENSION OF THE ARTERIAL WALL

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Submitted for publication February 28, 1940

The purpose of this communication is to describe the occurrence and cure of an arterial lesion not hitherto recorded.

W. M. (Unit 173429), a 48 year old white farmer, entered the hospital June 20, 1939, complaining of cramps in the muscles of the left leg, brought on by walking. His symptoms were of sudden onset and of two months' duration. After walking about four hundred yards he would develop a limp and severe pain in the calf of the left leg. These symptoms would disappear after a few minutes of rest, but would reappear when he walked again. Climbing one flight of stairs brought on the same symptoms. He had no other complaints, and said that he had always been healthy. His symptoms were no more severe at the time of admission to the hospital than they were at the beginning of his illness. The right leg was unaffected. He has always been a moderate user of tobacco and alcohol.

On examination his temperature, pulse, and respirations were normal. He appeared to be in excellent general condition, and no abnormality of heart, lungs, or abdomen was observed. There was no evidence of arteriosclerosis, and his blood pressure was 122/78 mm. of mercury. The left leg was slightly atrophic in appearance, and in measurement the circumferences of thigh and calf were slightly less than on the right side. No arterial pulsation could be felt anywhere below the inguinal ligament on the left side, although the pulsation of the left external iliac artery was plainly felt, and all arterial pulsations were easily palpated in the right leg. A faint systolic bruit could be heard over the left femoral artery just below the inguinal ligament. In approximately this same location, in the

left femoral space a small, deep-seated mass could be felt. This was rubbery, non-tender, not easily movable, and did not pulsate. The mass was about one centimeter in greatest dimension, and was thought to be a lymph node. The left foot was definitely pale, and was cooler than the right foot.

The results of clinical and chemical blood studies were normal, including a negative Wassermann reaction. The urine was normal. The electrocardiogram showed no evidence of cardiac disease. Roentgenograms of leg and pelvis did not show any arterial calcification.

We felt certain that the patient had had an occlusion of the femoral artery, and that it might be due to a neoplastic or granulomatous mass around the vessel, especially since there was no evidence of cardiac or general arterial disease. Thus hoping to find some remediable local condition, we decided to explore the artery.

On June 23, 1939, under spinal anesthesia the left common femoral artery was exposed. Upon retracting the inguinal ligament upwards the external iliac artery was seen to be normal in size and was pulsating well. The first portion of the common femoral artery was enlarged for a distance of 3 centimeters, and this enlargement felt rubbery and solid, and was without pulsation. Below the enlargement the artery appeared and felt normal, but was not pulsating. The femoral vein was not remarkable. The artery was carefully isolated and rubber-shod clamps were placed above and below the enlarged portion. The artery was incised longitudinally. To our surprise, as soon as the muscular coat was divided a quantity of light yellow gelatinous material oozed forth. This looked like myxomatous tissue. When this had been evacuated (perhaps 1.5 cubic centimeters) we could see the intima of the artery which was intact. The gelatinous material evidently was enclosed within the vessel wall outside the intima. The cavity was irrigated carefully with normal saline solution. The outer wall of the artery was then closed by means of a continuous silk suture, care being taken not to pierce the intima. The clamps were then removed and pulsation was now observed throughout the length of the artery. Examination directly after operation revealed a warm left foot with strong pulsations in the dorsalis pedis and posterior tibial arteries.

The patient convalesced uneventfully and was discharged on July

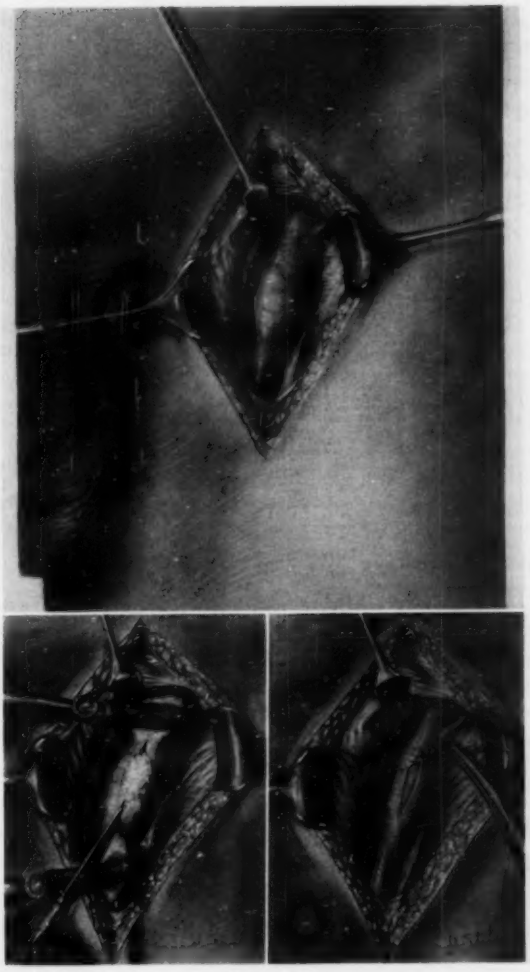

FIG. 1

Upper: exposure of femoral artery.

Lower left: incision of arterial wall and escape of gelatinous material from between media and intima.

Lower right: closure of femoral artery. Note that the intima was not incised.

11, 1939, symptom free. He had normal pulsations of the arteries of the left leg.

Fifteen days later the patient was readmitted with return of his original symptoms and signs. On the following day the artery was again explored. This time the occlusion was found to be due to dense scar tissue which had formed outside the vessel. Pulsation was re-established as soon as the scar tissue was excised. We now placed a pedicle fat graft about the artery, hoping to prevent excessive scarring. Again recovery was prompt and the patient left the hospital with normal arterial pulsations. He has been examined again, on February 9, 1940, more than six months after the second operation. He is still well, free of symptoms, and has good pulsations in the arteries of the left leg.

The material removed from the arterial wall was studied microscopically. It was considered to be coagulated plasma, containing neither cellular elements nor blood pigment.

DISCUSSION

This lesion is apparently a rare one, but the possibility of cure justifies exploration, in the future, of single arterial occlusions occurring in the absence of embolic phenomena or general arterial disease. The name for the condition was suggested by Dr. Rudolph Matas in a personal communication to the author. The etiology of the curious pathological condition in the arterial wall is obscure. It seems certain that the process was neither neoplastic nor infectious in origin. Aneurysms in this region have occasionally been described as being due to trauma to the vessel resulting from the anatomical position of the artery beneath the inguinal ligament; and some have thought that a cystic degeneration in the media of the arterial wall is the first stage in the formation of the aneurysm. If this is so, the present case may represent the cure of a pre-aneurysmal lesion. This is pure speculation, however. Occlusion of the vessel has not been described in those patients in whom aneurysm developed. It has also been suggested that this lesion may have been an early, or perhaps a healing, dissecting aneurysm. Without having the arterial wall to study, we are in no position to pass final judgment on the nature of the pathological process.

BOOK REVIEWS

(The reviews represent the individual opinions of the reviewers are not necessarily those of the members of the Editorial Board of this Journal.)

Gonorrhea in the Male and Female. By P. S. PELOUZE. 3rd ed., revised. 489 pp. Illus. $6.00. W. B. Saunders Company, Philadelphia, 1939.

The only real change in this standard textbook on gonorrhea has been the addition of newer knowledge of the work on culture of gonococcus and a discussion of chemotherapy. The author perhaps puts too much reliance upon the oxidase test in identifying the gonococcus. In a discussion of the newer chemotherapy he acknowledges that sulfanilamide is the greatest single advance in the treatment of gonorrhea, but at the same time gives a great deal of space to critical discussion in which the disagreeable features are perhaps over-emphasized. He quotes instances in which the female becomes infected without symptoms and attributes this to morphological changes in the gonococcus caused by sulfanilamide. This may be true but it is far from proven and may have occurred even before the days of sulfanilamide. The author lays great stress upon the determination of cure following chemotherapy. This is correct, but his discussion leads one to believe that the question of cure of gonorrhea is fairly simple in cases not treated with sulfanilamide. The difficulty of writing on the modern treatment of gonorrhea by chemotherapy, is obvious because many allied compounds are being used and each month brings forth more knowledge concerning many new compounds.

Dr. Pelouze's book is just as valuable to the clinician as ever. It still emphasizes a thorough knowledge of fundamentals concerning gonorrheal infection and should be read and heeded by all who attempt to treat this disease.

S. A. V.

Post Mortem Appearances. By JOAN M. ROSS. 4th ed. 275 pp. $2.50. Oxford University Press, New York and London, 1939. (Oxford Medical Publications.)

To the fourth edition of this book describing the various gross pathological changes one meets, the author has added a new chapter on still-births and neonatal deaths. This is clearly a welcome adjunct and should help focus attention on a subject which has received insufficient emphasis.

R. H. F., Jr.

You Can't Eat That! By HELEN MORGAN. 330 pp. $2.50. Harcourt, Brace and Company, New York, 1939.

This is a book which "internists, dietitians, sufferers from food sensitiveness, and those who cook for these sufferers will welcome."

In the preface, Dr. Walter C. Alvarez discusses the importance of keeping food diaries and suggests a simple method that is practical.

Miss Morgan divides her material into three sections. Part one deals with "Allergy and Allergics" which undoubtedly will interest the layman more than the doctor. Part two gives some excellent "Recipes" which may do much to dispel the monotony of the so-called elimination diet. Miss Morgan undoubtedly saved the best for the last for Part three, "Food Information for Allergics" deals with "Whats in it." This list gives the ingredients of each food and should prove a boon to the allergic person with a limited knowledge of food products and their preparation.

Miss Morgan's book will be useful to many groups, but especially to the patient whose doctor has told him "You Can't Eat That."

E. R. T.

An Introduction to Sociology and Social Problems. A Textbook for Nurses. By DEBORAH MACLURG JENSEN. 341 pp. $2.75. *C. V. Mosby Company, St. Louis, 1939.*

This book has been written as a text for two courses recommended for inclusion in the curriculum of Schools of Nursing by the National League of Nursing Education. The first section on Sociology, forming two-thirds of the book, is composed of five parts beginning with Man's Social Nature and the Development of Personality, followed by collective behavior, the community, the family and social change. This portion of the book attempts to cover a very wide range of subjects, and although well-arranged, they are dealt with so often in generalities that something is lost in interest and in meaning as applicable to the teaching of nurses.

The second section deals with the social problems related to illness, first as the individual's reaction to illness and then as the social problems in the modern community. This section of the book sets forth many topics concerning the social aspects of illness which should be considered essential to the education of nurses, especially those who enter the field of public health. The extensive use of quotations by the author tends to make the book more of a compilation and less of a text-book based on actual experience, but on the whole the book may prove useful in stimulating interest in teaching the social aspects of professional nursing.

G. C. R.

Personal and Community Health. By C. E. TURNER. 5th ed. 652 pp. Illus. $3.00. *C. V. Mosby Company, St. Louis, 1939.*

While this book is designed primarily for use in college courses in hygiene, it is of real value to the general reader who desires information about his own health and that of his community.

The material is, in general, sound and the manner of presentation is good though not exciting. It is not evident, however, just why the appendix containing tech-

nical details of the control of communicable disease, rare and common alike, should have been included. Its omission would reduce the size of the volume without affecting its value.

A. W. F.

Principles of Chemistry. By JOSEPH H. ROE. 5th ed. 503 pp. Illus. $3.00. *C. V. Mosby Company, St. Louis, 1939.*

This reviewer must leave to others a judgment of the wisdom of including in one text treatments of general, inorganic, organic, and physiological chemistry together with laboratory experiments. Since the author has designed this book for nurses and students of home economics one may guess that the scope of content has been determined in the first instance by administrative requirements. What seems to have been attained is not so much the essential unity of the several branches of chemistry as a simplicity of statement that doubtless serves the purpose well.

W. M. C.

An Introductory Guide to Biochemistry. By SIDNEY BLISS. 103 pp. $1.25. *W. B. Saunders Company, Philadelphia, 1939.*

The author states that his aim is modest. It is to introduce the reader to various subjects by means of greatly simplified statements.

Statement of the subject matters of thirteen large topics, crowded into eighty-six pages, necessarily leaves unmentioned many matters that have the greater interest and the greater value. On the wisdom of the choices made there can be difference of opinion but criticism on this score should rest with the reader. Many will find the book suggestive.

Legitimate criticisms are: (1) the inadequacy of several chapters leaves the reader about where he began, (2) inaccuracies. Illustrating the first is the chapter of less than two pages on Chemotherapy most of which says little. Illustrating the second is the statement that a fat is a salt.

W. M. C.

The State and Medical Research. By SIR EDWARD MELLANBY. 52 pp. 3/6 net. *Oliver & Boyd, Edinburgh and London, 1939.*

This small volume is the Harveian Oration delivered before the Royal College of Physicians on October 18, 1938. The author is the secretary of the Medical Research Council of Great Britain and most of the lecture, after an initial tribute to Harvey, is devoted to an exposition of the policies which govern that body and a comprehensive description of its varied activities.

Support of research by Parliament is justified by the author on the ground that in Great Britain private endowment was altogether inadequate for the purpose. He then gives an account of the extent of government support of medical research and of the measures adopted by the Medical Research Council to avoid the pitfalls of "repressive control" and "bureaucracy" so often associated with

governmental enterprises. The author concludes with the statement that the principal difficulty is not so much the acquisition of knowledge as its application to human needs, and proceeds to cite specific instances where such application lags in Great Britain as contrasted with other parts of the Empire.

The book should be of particular value to those who are charged with the disbursing of funds for medical research, whether their source is governmental or private.

A. M. C.

Architecture of the Kidney in Chronic Bright's Disease. By JEAN OLIVER. 257 pp. Illus. $10.00. *Paul B. Hoeber, New York, 1939.*

This volume with its numerous and beautiful illustrations is an amplification and extension of work previously published in part in the Archives of Pathology. It sets forth in detail the conclusions which may be deduced from a very special form of anatomical study, as to the pathological changes in the kidney which take place during the progress of Bright's disease. The methods that have been employed are microscopical dissections and reconstructions of the architectural unit of the kidney or nephron. In general terms the nephron is found to undergo changes that lead either to an hypertrophied glomerulotubular unit, or, through regressive changes, to an atrophied glomerulotubular unit. The complicated histological picture of the kidney in advanced Bright's disease may be explained by the predominance of one or the other of these processes or of intricate combinations of the two. The author further attempts to explain by the employment of this technique the mechanism by which hypertrophy or regression and atrophy of the unit is brought about. The discussion of these problems is converted into an objective study through the use of remarkable and beautiful pictures with which the volume is richly illustrated. The full-page line drawings of reconstructed units, and the photomicrographs of sections of diseased kidneys are excellently done, but one cannot but help admire extravagantly the thirty-some plates reproducing stereograms of the dissected nephrons that can only be regarded as works of art. Not only is the author to be congratulated on the reconstruction of these dissections and models, but the publishers on their admirable reproduction of them.

W. T. L.

Urology. By DANIEL N. EISENDRATH AND HARRY C. ROLNICK. 4th ed., revised. 1061 pp. Illus. $10.00. *J. B. Lippincott Company, Philadelphia, 1938.*

The authors of this popular text on the essentials of urology have successfully brought the fourth edition up to date through a wide revision based largely on an exhaustive survey of the world's literature. The work is encyclopedic and practical, and the subject matter is well organized and readable. A carefully arranged index renders the information readily accessible.

In places the volume seems a bit bulky through the inclusion of a certain amount of material of rather remote significance. Elsewhere, classification of cases ac-

cording to variations in symptomatology places disproportionate emphasis upon this aspect of the clinical picture and serves to confuse rather than to clarify. Occasionally the personal opinions of the authors upon controversial matters are stated somewhat dogmatically.

The volume should prove especially useful to both students and practitioners, who should find the logical arrangement of the comprehensive material and the 750 illustrations and diagrams very helpful.

H. J. J.

Medical Jurisprudence and Toxicology. By WILLIAM D. MCNALLY. 386 pp. Illus. $3.75. *W. B. Saunders Company, Philadelphia, 1939.*

A comprehensive, but brief, review of toxicology is presented. It includes a discussion of many of the recently recognized industrial poisons. Criminological investigations are outlined. The discussion of medical jurisprudence deals, for the most part, with the legal responsibilities of physicians. Medical jurisprudence as related to autopsy study, is inadequately presented, and conclusions therefrom are unsatisfactory.

W. B. V.

John Howard (1726–1790), Hospital and Prison Reformer: A Bibliography. By LEONA BAUMGARTNER. 79 pp. Illus. $1.00. *The Johns Hopkins Press, Baltimore, 1939.*

All persons interested in the life and works of John Howard owe a debt of gratitude to Dr. Leona Baumgartner for her exhaustive and critical bibliography of Howard's writings and the far greater number of books and articles that have been written about him. This bibliography was first published in two issues of The *Bulletin* of the Institute of the History of Medicine, appearing in 1939. Its publication in book form makes it more readily available for students of Howard.

The book very fittingly begins with an introduction by Arnold M. Muirhead, a well-known collector of Howardiana who gives a brief biographical sketch of Howard and critical comments on his published works.

The bibliography itself lists first the various editions of Howard's two major works—The State of Prisons in England and Wales, and An Account of the Principal Lazarettos in Europe. Following this is a list of his less well-known writings. The rest of the book which lists the various biographies, biographical sketches and commentaries on the character and work of Howard is especially valuable not only on account of its completeness, but because of the fact that Dr. Baumgartner briefly and concisely evaluates them.

J. E. R.

BOOKS RECEIVED FOR REVIEW

Introduction to Physiological and Pathological Chemistry, with Special Reference to the Needs of Schools of Nursing. By L. EARLE ARNOW. 555 pp. Illus. $3.50. C. V. Mosby Company, St. Louis, 1939.

Psychobiology and Psychiatry. By WENDELL MUNCIE. 739 pp. Illus. $8.00. C. V. Mosby Company, St. Louis, 1939.

Ophthalmology. By BURTON CHANCE. 240 pp. Illus. $2.00. Paul B. Hoeber, Inc., New York, 1939. (Clio Medica xx.)

Bacteriology. By WILLIAM W. FORD. 207 pp. Illus. $2.50. Paul B. Hoeber, Inc., New York, 1939. (Clio Medica xxii.)

The Flowering of an Idea. A play presenting the origin and early development of The Johns Hopkins Hospital. By ALAN M. CHESNEY. 87 pp. $1.50. The Johns Hopkins Press, Baltimore, 1939.

Psychopathia Sexualis. By RICHARD VON KRAFFT-EBING. Only authorized English adaptation of the last German edition revised by Kraft-Ebing. 626 pp. $3.00. Pioneer Publications, Inc., New York, 1939.

Das Hospital zu St. Georg in Leipzig durch acht Jahrhunderte, 1212–1940. By C. SEYFARTH. 1. Band. 249 pp. Illus. RM. 10 (paper); RM. 11 (bound). Georg Thieme, Leipzig, 1939.

Cancer of the Larynx. By CHEVALIER JACKSON AND CHEVALIER L. JACKSON. 309 pp. Illus. $8.00. W. B. Saunders Company, Philadelphia, 1939.

Manual for Diabetic Patients. By W. D. SANSUM, ALFRED E. KOEHLER, AND RUTH BOWDEN. 227 pp. Illus. $3.25. The Macmillan Company, New York, 1939.

Tumors of the Hands and Feet. Edited by GEORGE T. PACK. 138 pp. Illus. $3.00. C. V. Mosby Company, St. Louis, 1939.

Essentials of Medical Treatment. By DAVID MURRAY LYON. 448 pp. Illus. 15 shillings. Oliver & Boyd, Edinburgh, 1939.

Vitamin D: Chemistry, Physiology, Pharmacology, Pathology, Experimental and Clinical Investigations. By C. I. REED, H. C. STRUCK, AND I. E. STECK. 389 pp. $4.50. University of Chicago Press, Chicago, 1939.

Industrial Hygiene. By various authors. Edited by A. J. LANZA AND JACOB A. GOLDBERG. 743 pp. Illus. $8.50. Oxford University Press, New York, etc., 1939.

Lehrbuch der Augenheilkunde. By ERNST FUCHS. 16th ed. Revised by Dr. ADALBERT FUCHS. 904 pp. Illus. Paper, 27 marks; bound, 30 marks. Franz Deuticke, Wien, 1939.

LUNG ABSCESS

Analysis of 88 Cases

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Submitted for Publication January 24, 1940

The continued high mortality of lung abscess is a constant challenge to the medical profession, and in spite of the advances in many other fields, the treatment of this condition remains far from satisfactory.

The purpose of this report is to analyze the more important features in a group of 88 patients with lung abscess, with special emphasis on the results of the various types of treatment employed, and with suggestions as to the most efficient therapeutic approach. Sixty of the cases were from the records of The Johns Hopkins Hospital during a period of eight years, from 1931 to 1938, and 28 were from the Union Memorial Hospital during approximately the same interval.

We have excluded from this study lung abscesses which have arisen as complications of lung tumors; those definitely originating from a long-standing bronchiectasis; all cases in which tuberculosis might have been a complicating factor; and multiple embolic abscesses occurring in the course of a generalized septicemia.

No effort has been made to separate the cases of pulmonary gangrene from those of abscess, for it seems to us that no clear-cut dividing line can be drawn between them in the majority of instances. There is a certain amount of necrosis or gangrene associated with almost all lung abscesses sooner or later, no matter what the original infecting organisms may have been; and clear-cut, isolated cases of one or the other are hard to find. It is true that in some of the more acutely ill patients the sputum is very foul, thin, often dark with blood and contains many fusiform bacilli and spirochetes; whereas in other patients who often do not seem very ill, there is only a small amount of purulent sputum which does not have a foul odor. The former type of disease might

be referred to as "pulmonary gangrene," and the latter as "lung abscess," but inasmuch as it is rare to find them so sharply differentiated, we feel that it is less complicated if we group them all together as lung abscesses.

The literature will not be reviewed in any detail for there have been excellent summaries in recent years by Maxwell (31), Allen and Blackman (3), Kline and Berger (27), Cutler and Gross (14), Freedman (20) and others. Some of the more significant contributions will be briefly considered, however, in connection with the various headings which are to follow.

TABLE I

Age and sex of 88 patients

3 to 20 years	8 cases
21 to 30 years	25 cases
31 to 40 years	16 cases
41 to 50 years	14 cases
51 to 60 years	22 cases
61 years and above	3 cases
Average age successful cases	39 years
Average age unsuccessful cases	42 years
Males	58 cases
Females	30 cases

CLINICAL FEATURES

Age and sex

All the patients in this study were adults except five children whose ages ranged from 3 to 6 years. The records in the pediatric departments were not investigated. The ages are shown in Table I.

Duration

The duration of the abscess is of great importance, for whereas many of the patients get well spontaneously if the duration prior to admission is less than two months, the percentage of unsatisfactory results rises rapidly and progressively thereafter. Table II illustrates this point, and also shows that the time elapsing between the onset of symptoms and admission to the hospital is much too long. The

average duration of those whose outcome was unsatisfactory was twice that of those with good results. This is in accord with the experience of others. King and Lord (25) have emphasized the fact that a short duration is one of the most important factors influencing spontaneous recovery. Freedlander (19) stated that of those having symptoms for less than two months 66 per cent improved, whereas in those whose symptoms lasted longer than two months, not more than 31 per cent improved. This, in general, represents the view held by the majority of workers in this field, and our experience substantiates this in a striking way.

TABLE II

Duration prior to treatment

	months
Successful cases	1.8
Unsuccessful cases	3.6
Average for whole series	2.6

Not listed under the above successful cases are one of 3 and one of 8 years duration, while one case of 4 and one of 7 years duration were not included among the unsuccessful cases.

Location of abscess

There is a general belief that most lung abscesses occur in the lower lobes. This is used as one of the arguments in favor of the aspiration theory of the etiology of lung abscess. From a consideration of the numerous reports in the literature this belief seems justified. However, it is not always supported, for there are some series of cases, including our own, in which the upper lobes are found to be more frequently involved than the lower. For instance, in the 172 cases reported by Flick et al. (18), the upper lobes were more frequently involved than the lower lobes. Cutler and Gross (14) found the largest number in the right upper lobe, and the next largest in the left upper lobe. Freedlander (19) pointed out that the most common sites for abscess to begin are the apex of the lower lobe posteriorly and the posterior tongue of the upper lobe. In Lueth and Sutton's (29) 101 cases the greatest number were in the middle and lower thirds of the right lung. In our own cases, the distribution was that shown in Table III.

Etiology and pathogenesis

The controversy as to the etiology of lung abscess has raged for many years and, as yet, no entirely satisfactory conclusions have been reached. In general, there are two main schools of thought: one of these claims that the abscess is initiated by ***aspiration***, while the other holds that ***embolism*** is the first step in abscess production. The arguments have centered mainly around post-operative abscesses and in particular those following tonsillectomy—the operation which has preceded a high percentage of the cases reported in the literature. The champions of the embolic theory, in particular Cutler (13), Cutler and Gross (14), and Schleuter and Weidlein (41), point out the great difficulty in experimental production of lung abscess by the

TABLE III

Location of abscess

Right upper lobe	21
Right middle lobe	11
Right lower lobe	14
Left upper lobe	14
Left lower lobe	19
Left upper and left lower lobes	6
Bilateral, lower lobes	2
Multiple lobes	1

bronchial route, its occurrence following local as well as general anesthesia, and the rather long latent period between the operation and the onset of symptoms of abscess. Moreover, they have been able to produce these lesions in dogs rather consistently by the use of infected emboli. These abscesses have a marked tendency to heal quickly, however, unless the clot be infected with pyorrhea scrapings. In the latter case, chronic abscesses are formed containing various anaerobic and aerobic organisms. The frequent involvement of the upper lobes in their clinical cases was taken as another argument in favor of embolism, but it has been shown that emboli are more apt to lodge in the lower lobes in man, and in Schleuter and Weidlein's series of dogs the emboli also had a predilection for the lower lobes. On the other hand, in spite of the fact that a dog is not a good animal in which to attempt to produce abscesses *via* the bronchi (because of the high

degree of resistance of the dog's lung to infection and because of the more or less horizontal position of the tracheo-bronchial tree, allowing better drainage), successful attempts have been made in this way in the experimental production of lung abscess. The most constant results have been obtained by introducing an inoculum made up of pus from lung abscesses or from advanced cases of pyorrhea—such pus containing a variety of organisms including spirochetes, fusiform bacilli, anaerobic or aerobic cocci and Gram-negative bacilli. This material is then trapped in the bronchi by some mechanical means, thus blocking the bronchus and producing an area of atelectatic lung tissue whose resistance to infection is much lowered. In this manner, with a heavily infected inoculum, and with drainage temporarily cut off, chronic abscess may be produced in the atelectatic area in a small proportion of cases. A healthy animal's lung is very resistant to infection and the main lines of defense are the cough reflex, the intact cilia, the normal flow of mucus, and the activity of the macrophages or wandering cells. The same defense mechanisms are active in the human being, and that no doubt explains the infrequent development of aspiration lung abscesses in spite of the very frequent occurrence of aspiration of infected material into the lower bronchial tree, just as it explains the difficulty of their experimental production. The following are a few of those who have helped to clarify this aspect of the problem: Crowe and Scarff (12), Allen (2), Hedblom *et al.* (22), D. T. Smith (43), Harkavy (21), Weiss *et al.* (50), Weiss and Shevky (51).

Those favoring the aspiration theory of the etiology of lung abscess also point out the increased frequency of its occurrence when the tonsils, tooth sockets or gums are heavily infected, and when deep ether anesthesia is used, thus favoring an overproduction of mucus and an abolition of the cough reflex. They also mention the decrease in its frequency when the patient's head is kept lowered throughout the operation. In this connection the following figures as given by Crowe (11) are of extreme interest and importance. Since 1912 there have been approximately 20,000 tonsillectomies at The Johns Hopkins Hospital without record of the development of a single lung abscess as a result of this operation. The important points in the operative technique are as follows: (1) morphine is not used and the anesthesia is kept light; (2) the patient's position is supine with the

feet eighteen inches higher than the head; (3) a suction tube is used throughout to keep the throat clear; (4) sharp dissection and careful clamping are employed. The importance of careful hygiene pre-operatively is also stressed. The remarkable results reported by Crowe lend strong support to the aspiration theory of the etiology of post-tonsillectomy lung abscess.

The ease with which aspiration can occur under light anesthesia, local anesthesia, and even during quiet sleep has been shown by many workers. This knowledge has focussed attention more and more on the importance of infected teeth, gums, tonsils and sinuses in the pathogenesis of pulmonary infections. Among those who have contributed to this phase of the study is Myerson (34), who broncho-scoped 100 patients just after tonsillectomy and found blood or mucus in the lower bronchi in all but 6 of 78 whose cough reflex was absent, but in a much lower percentage of those with cough reflex present. Lemon (28) observed aspiration in dogs and showed the marked influence of the plane of the dog's body on the occurrence of aspiration into various parts of the bronchial tree. Faulkner and Faulkner (17) found that if lipiodol were put into the nose during sleep, it could be demonstrated in the bronchi the next morning. Their article on "internal drainage" of the lungs dealt mainly with post-operative atelectasis. It showed how the position of the patient, during and after operation, will often determine the site of pulmonary atelectasis; and it stressed the great importance of pre-operative oral hygiene, the Trendelenberg position after operation, and inhalations of CO_2, in preventing pulmonary complications. The observations and experiments of Joannides (24) serve to stress this same point. Bettman (4) found the same to be true with dogs under general anesthesia. He put a few drops of gentian violet in the back of dogs' mouths before operation and noted in practically every instance that both lungs were deeply stained with the dye when the animal was sacrificed after operation.

According to the theory of Van Allen *et al.* (45), the most common cause of lung abscess is a combination of embolism and aspiration. They successfully employed this combination in dogs by using emboli infected with staphylococcus aureus, and then introducing, *via* the bronchus, organisms obtained from lung abscess sputum. Cutler (13) found that if the emboli he used were infected with ordinary

pyogens, only a temporary infection resulted, but chronic abscesses were formed when the clot used as an embolus was infected with organisms from pyorrhea scrapings. It is also recognized clinically that infarcted lung tissue is very susceptible to infection and in cases of poor oral hygiene there is an ideal opportunity for this to occur by aspiration.

Probably the most important single step in the pathogenesis of the majority of lung abscesses, however, is *atelectasis*. This may follow the plugging of a bronchus or bronchi, post-operatively, by thick mucus or blood; it may occur in the course of pneumonia if bronchi are plugged by tenacious exudate; it may follow the aspiration of vomitus or a foreign body. The probable mechanism of many cases of post-operative atelectasis and pneumonia has been ably discussed by Coryllos and Birnbaum (10), who claim that most cases of pulmonary consolidation are actually preceded by atelectasis, which has resulted from bronchial obstruction by tenacious mucus. If virulent pneumococci were in the bronchial secretions, lobar pneumonia resulted, but if anaerobic organisms of the mouth were present, a lung abscess or gangrene might result. This viewpoint is especially pertinent, because we know how difficult it is to infect normal lung tissue, whereas atelectatic lung tissue has lost a great deal of its resistance to infections of all types. The probable importance of this mechanism in the etiology of lung abscess has been stressed by many authors in recent years.

The more important conditions observed by various authors to precede the development of lung abscess are summarized in Table IV, along with our own data. The large number of cases in which lung abscesses have followed operations involving the upper respiratory tract, especially tonsillectomies, is of course well known. King and Lord (25), in a series of cases observed in the years 1924–1932, found that 1 out of every 2 followed an operation upon the upper respiratory tract. Maxwell (31), on the other hand, reported a very low incidence associated with tonsillectomies, most of the lung abscesses following pneumonia or abdominal operations. Freedman (20) believes that the importance of tonsillectomies is much exaggerated and that the reason more abscesses occur after this operation than after others, is because so many more of these operations are performed.

It is difficult in a great many of our patients to be sure of the

etiology, for an overwhelming majority developed outside the hospitals, and our impression as to the probable cause of the abscess had to be derived chiefly from the history. However, it is interesting to observe the high percentage of cases which followed respiratory infections, most of which were probably pneumonia.

TABLE IV

Outstanding conditions preceding onset of lung abscess

From several series of cases. Approximate figures

	ALLEN AND BLACKMAN, 1936	CUTLER AND GROSS, 1936	LUETH AND SUTTON, 1936	WARNER, 1938	FREEDMAN, 1938	FISHER AND FINNEY (PRESENT SERIES)
Number of cases	94	90	101	98	276	88
1. Pneumonia (broncho, lobar or aspiration pneumonia)	18%	28%	13%	20%	38%	40%†
2. Upper respiratory tract infection (chest colds, bronchitis, influenza, etc.)			30%		26%	9%
3. Operations in upper respiratory tract (mainly tonsillectomy and dental extraction)	24%	39%	10%	30%	10%	15%
4. Operations in other parts of the body (mainly abdominal operations)	10%	14%			15%	13%
5. *Unknown* (preceding condition not definite. First symptoms usually those of lung abscess)	38%*	14%	30%	33%		10%

† Miscellaneous causes accounting for 13.5% are not included in this summary.

* From the author's description many of these would probably be classified under "upper respiratory infections" above.

Bacteriology

This is a complicated chapter in the study of lung abscess, for the findings vary greatly depending upon the methods and the care of the different workers. In the majority of the cases many different bacteria are present in the sputum, in pus obtained by bronchoscope, and even in cultures taken directly from the abscess. It is difficult to determine which organisms are the important ones: which are primary, and which are secondary invaders. In the sputum itself there are usually aerobes, facultative anaerobes and often strict anaerobes, though it is hard to isolate the latter since they are apt to be over-

grown by the aerobes. In actual cultures from the abscess at operation or autopsy, and from the pleural cavity in cases in which there has been rupture of the abscess, the cultures reveal mainly *anaerobes*. Some of these are strictly anaerobic, others are facultative, and still others, which are first isolated as strict anaerobes, after repeated transplantation grow also under aerobic conditions. These bacteria are presumably aerobes which have become anaerobic through their adaptation to the environment of walled-off chronic abscesses. Ermatinger (15) in particular has drawn attention to this change in their biological properties. The usual similarity of the flora found in lung abscesses to that of the mouth, especially that about infected gums, has been repeatedly remarked upon. It is difficult to tell whether or not it is necessary to have several varieties of organisms growing together in the lung tissues in order to produce a lung abscess. It seems quite likely that this symbiosis is important, because the few times when lung abscesses have been produced experimentally, material containing several different types of bacteria, or bacteria and spirochetes, has been used in the inoculum.

The importance of the combination of spirochetes and fusiform bacilli is another controversial subject. D. T. Smith (43, 44) who stresses the association of these organisms with anaerobic streptococci and vibrios, has found them in the walls of abscesses, pathologically. He has also produced abscesses experimentally in animals with this combination of organisms. Kline and Berger (27), Pilot and Davis (40), and others have found a high percentage of spirochetes, fusiform bacilli and other bacteria in their material. The rapid clearing of some lung abscesses under treatment with neoarsphenamine is additional evidence of the significance of these organisms, but this is not a common result except in the acute cases in which the sputum is teeming with spirochetes. Many others believe that the rôle of the spirochete and fusiform bacillus is that of secondary invaders; that in those cases in which they are present in considerable number they may be important factors in keeping the abscess chronic; and that they probably contribute to the foul odor of the sputum. In many cases of lung abscess the odor of the sputum and breath becomes less offensive after injections of neoarsphenamine, although the course of the disease may not be materially affected.

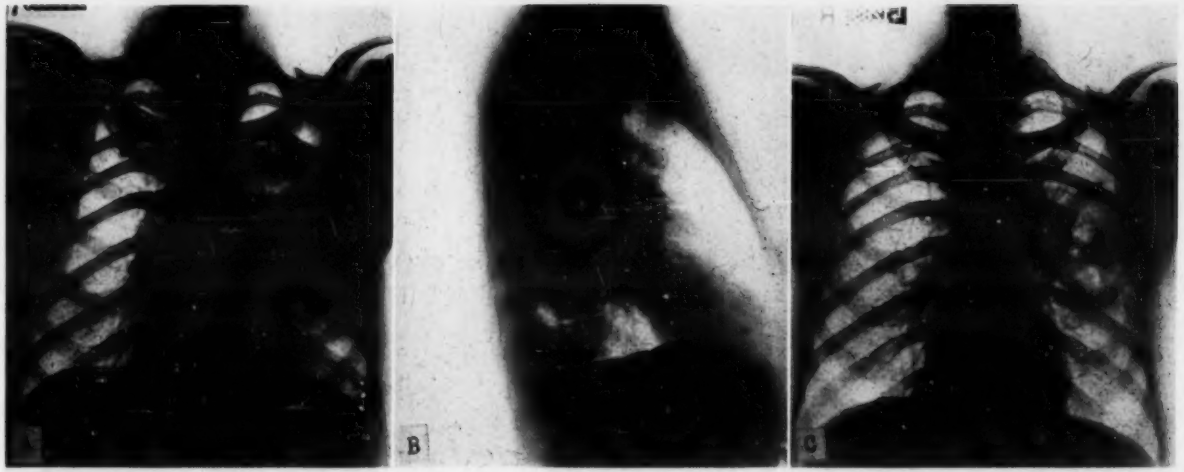

FIG. 1. AN ABSCESS OF 3½ WEEKS' DURATION WHICH CLEARED UP RAPIDLY AFTER SURGICAL DRAINAGE

E. S., male, white, aged 38 years. Duration of abscess prior to admission: 3½ weeks. Onset following tonsillectomy. Treatment: Two-stage operation 4 days after admission: (1) rib-resection with insertion of gauze pack, (2) two days later cautery drainage. Result: Complete recovery. Entirely well at last report late in 1939.

Photographs: (a) September 13, 1934 (on admission): Extensive lesion with large cavity. (b) September 13, 1934: Lateral view of same. (c) October 2, 1934 (16 days post-operatively, 7 days before discharge): Condition much improved but lung not yet entirely clear.

Varney's (46) careful studies of sputum and pus revealed a large percentage of anaerobic Gram-negative bacilli which, when cultivated on human blood agar, grow out after 5–6 days inoculation and produce a foul odor and dark brown pigment. This bacillus, B. melaninogenicum, was first described by Oliver and Wherry (38) in 1921. It was found by Burdon (8) in various tissues in symbiosis with other organisms, and by Schwarz and Dieckman (42) in blood and uterine cultures of patients with puerperal infection. In an excellent summary of the subject, Cohen (9) describes methods for the bacteriological study of lung abscesses, and remarks especially on the frequent association of gamma streptococci and B. melaninogenicum in symbiosis in these infections. Other bacteria were found also, mostly anaerobes. Others who have made important contributions to this subject are Allen and Blackman (3), who found anaerobes in 80 per cent of their cases, Miller and Lambert (32), Bucher (7), Marshall and Brunn (30) Weiss and Mercado (49), Kline (26), and others.

Bacteriological studies in the present series of cases

In our own patients satisfactory cultures were obtained from the sputum or by bronchoscope in 60 instances, from the abscess itself in 26 instances, and from empyema fluid in 9. A summary of the findings is shown in Table V.

The organism most commonly appearing in predominant or significant numbers in the sputum and in the pus obtained by bronchoscope, or from the abscess itself, was the aerobic *alpha hemolytic* (viridans) *streptococcus*, which was cultivated in 36 cases.

From the abscesses themselves, in a limited number of cultures, *anaerobic* and *facultative anaerobic* organisms predominated, and of these, 8 were *streptococci*. However, many other organisms were usually found in smears of the pus, the most common among them being Gram-negative bacilli. These were sometimes isolated as "anaerobic Gram-negative bacilli" (5 of our cases), but they were more often not actually isolated on culture media because of the difficulty of their cultivation. It is likely that many of these were B. melaninogenicum, for others have found this organism frequently with special methods of cultivation. No significant difference was found in the bacterial flora of the abscesses in the fatal cases as compared with the cultures

TABLE V

I. Cultures of sputum or of exudate obtained by bronchoscope in 60 cases (in order of the frequency of their appearance in significant numbers)

(1) *Aerobic cultures:*

	number	*per cent*
1. Alpha hemolytic (viridans) streptococcus	29	48
2. Pneumococcus	21	35
3. Staphylococcus aureus (usually hemolytic)	18	30
4. Staphylococcus albus	11	18
5. H. influenzae	9	15
6. Beta hemolytic streptococcus	7	12
7. Gamma (non hemolytic) streptococcus	7	12
8. Neis. catarrhalis	6	10

Other bacteria include diphtheroids, B. coli, H. hemolyticus and others.

(2) *Anaerobic cultures:*

1. Alpha hemolytic streptococci (facultative anaerobes)	8
2. Anaerobic streptococci (strict anaerobes)	2
3. Anaerobic Gram negative bacillus	1

(3) Spirochetes (in smears)	22
Fusiform bacilli (in smears)	13

II. Direct cultures from abscesses (at operation or at autopsy) ..... 26 cases

1. Alpha hemolytic streptococcus	9 (2 of these were facultative anaerobes, the rest aerobes)
2. Anaerobic streptococcus	6
3. Staphylococcus aureus	6
4. Anaerobic gram negative bacillus	5
5. Pneumococci	5
6. H. influenzae	4
7. Other staphylococci	4
8. Hemolytic streptococcus	2
9. B. coli	2
10. Spirochetes (in smears)	5
11. Fusiform bacilli (in smears)	5

Various other organisms were found in fewer numbers

In one a Gamma streptococcus predominated. In another there were many branching organisms having the appearance of actinomyces.

III. Cultures of empyema fluid ..... 9 cases

1. Anaerobic streptococci	6
2. Anaerobic Gram negative bacilli	3
3. Anaerobic diphtheroids	2
4. Aerobic beta hemolytic streptococcus (pure culture)	2
5. Staphylococcus aureus	2
6. Fusiform bacillus (smear)	1

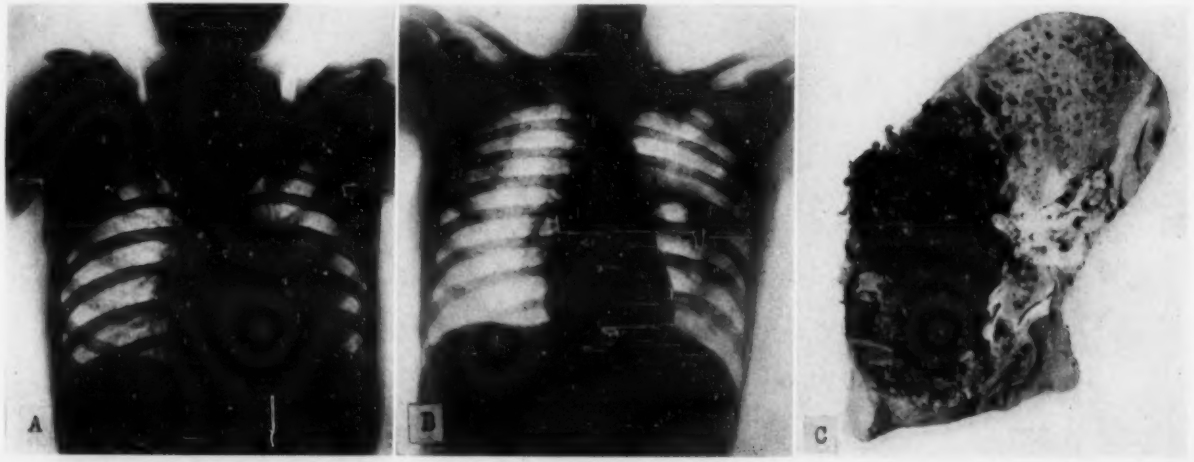

FIG. 2. AN EXAMPLE OF AN ACUTE OVERWHELMING TYPE OF INFECTION WITH PULMONARY GANGRENE. TEN OF THE FATAL CASES RAN COURSES MUCH LIKE THIS ONE

F. C., female, colored, aged 30 years. Duration of abscess prior to admission: 3 weeks. Onset following epileptic seizure with probable aspiration. Dental caries. Treatment: Postural drainage, neoarsphenamine, transfusion. Result: Died in 2 weeks with a rapidly spreading, destructive type of lesion. Autopsy showed large area of gangrene occupying most of the left lung, with abscesses and lobular pneumonia in both lungs.

Photographs: (a) October 10, 1935: Large cavity in left lung. (b) October 17, 1935: Showing extension of the process. (c) Left lung as seen in cross-section at autopsy. Large area of necrotic tissue in the lower lobe with consolidation in the upper.

from those which subsequently healed. Cultures taken from the pleural fluid in the patients with empyema yielded organisms which were essentially similar to those obtained from the lung abscesses themselves, except that the alpha streptococcus was not found and the percentage of anaerobes was higher. The probable importance of symbiosis of several varieties of microorganisms in these infections is further emphasized by two fatal cases in which there were metastatic abscesses. In one of these, lesions in the brain and pericardium as well as the lung, showed spirochetes, fusiform bacilli, Gram-positive cocci and Gram-negative bacilli. In the other case there were abscesses in many organs, and in all of them spirochetes, Gram-negative bacilli and Gram-positive cocci were found.

Symptoms and signs

The important clinical findings in cases of lung abscess are too well known to warrant any detailed review or analysis. In the series of 172 cases reported by Flick *et al.* (18), 99 per cent had cough, 45 per cent hemorrhage, 62 per cent fever and 71 per cent pain. The last of these they found to be the most common *early* symptom. Moderate secondary anaemia was common. Warner (47) found 80 per cent of his cases to have foul sputum and 54 per cent had blood in the sputum. In Lueth and Sutton's (29) review of 101 cases, 95 per cent had expectoration (usually profuse), 54 per cent pain in the chest, all had fever, 60 per cent showed dullness and 58 per cent rales. The important X-ray findings were successive changes from increased density, to sharper demarcation, then to cavity with fluid level. Allen and Blackman (3) likewise called attention to the importance of pain at the site of the abscess and the value of X-ray in accurate localization.

Cough is almost invariably present at some time during the illness and is usually the first symptom. It is dry at first but later becomes productive. The expectoration later becomes abundant, and often foul. The appearance of the sputum varies a great deal: it may be frankly purulent with little odor; muco-purulent and often blood streaked; or thin, watery, brownish and very foul. Frank hemoptysis occurs in about 20–40 per cent of cases. There is usually some fever, and chills are apt to occur at intervals during the illness. The frequent occurrence of pain, often "pleural" in character, in the region

of the abscess was mentioned above as an early symptom. It is also well to bear in mind the presence of this symptom when surgery is contemplated, for in such cases one would expect the pleural surfaces to be adherent in the affected region, thus making the abscess more amenable to surgical drainage.

Some of the outstanding physical findings are the following: an offensive breath (especially marked after a coughing spell); pyorrhea and dental caries, frequently present; dullness, suppressed breath sounds and rales over a small lung area; breath sounds, at times, bronchovesicular or tubular, may be amphoric when the cavity is large, close to the surface and relatively empty. It is well to remember, however, that it is common to have *no* pulmonary signs. It is likewise a common occurrence to observe marked changes in the pulmonary findings from day to day. For instance, on one occasion there may be no signs, on the next day rales or even signs of cavity, and a few days later no signs again. Clubbing of the fingers and toes, an important finding when present, is more apt to be found in the chronic cases. However, some chronic cases of several years' duration are seen, which show no clubbing, while in other cases clubbing may develop within two weeks of the onset of symptoms. Leucocytosis is more common in the acute and subacute cases, and anaemia in the chronic ones. The X-ray findings are apt to be inconclusive if only one or two films are taken. The most important X-ray changes during the evolution of the abscess are at first an ill-defined area of increased density, which later becomes more sharply demarcated, and in which there may finally develop a cavity with fluid level. The lesions can best be localized by taking the X-rays with the patient in various positions. Lipiodol injection is useful mainly in helping to localize the abscess and in ruling out foreign body, tumor, bronchiectasis, *etc.*; but the lipiodol rarely enters the abscess cavity itself.

The symptoms and signs in our own cases corresponded closely to the ones outlined above. The occurrence of gross hemoptyses was noted in 26 per cent and chest pain was a prominent symptom in 40 per cent. Sixty-five per cent showed profuse expectoration and in 60 per cent the sputum was described as having an offensive odor. The pulmonary signs were very meagre in many patients; the most common signs were dullness (43 per cent) and rales (40 per cent).

TREATMENT

A. Prophylaxis

The importance of the preventive aspect of therapy cannot be emphasized too strongly. This consists primarily in careful daily care of the teeth and gums, especially before any operations are contemplated, particularly those upon the upper respiratory tract. Secondly, in all operations it is very important to avoid as far as possible an irritating, deep and prolonged anaesthesia, which is likely to result in excessive production of mucus and abolition of the cough reflex long enough to allow aspiration into the smaller bronchi. If the aspirated material is viscid enough, it may produce bronchial obstruction with atelectasis; and if it contains organisms from the upper respiratory tract, secondary infection may result. The position of the patient during the operation should be such as to favor drainage toward the mouth rather than into the lower bronchial tree, whenever possible. Sedatives should not be given in large doses, for this results in depression of the cough reflex and promotes shallow breathing, thus favoring stasis of secretions in the lower bronchi. All secretions that develop in the large bronchi or trachea during the period of anaesthesia should be thoroughly aspirated with nasal catheter suction. As the patient wakes up, care should be taken to prevent aspiration of vomitus by keeping the foot of the bed elevated, or by further use of suction. Post-operative inhalation of carbon dioxide and oxygen has been recommended by Coryllos and Birnbaum (10), Faulkner and Faulkner (17), and others as a means of overventilating the lungs, thus preventing the stagnation of mucus and other material. In many clinics, including our own, this is carried out while the patient is on the operating table and every few hours thereafter for 2 or 3 days.

B. Medical treatment

1. General. The first and most important step in the treatment of the actual lung abscess is a comprehensive program of medical management. Following a careful diagnostic study a régime is instituted that aims at building up the patient's resistance, combating the infection, and attempting to secure drainage through the bronchi. This means, in most patients, rest in bed, a high-calorie, high-vitamin diet, plenty of fresh air and a high fluid intake. Steam inhalations

containing creosote and benzoin are often helpful – especially when the cough is severe and unproductive—in liquefying the secretions and in soothing the bronchial mucosa. Creosote also has the effect of rendering the odor of the breath and the sputum less offensive, whether it is used in inhalations or taken by mouth. Potassium iodide or ammonium chloride, by mouth, can likewise often be used to advantage for making the secretions less viscid. It is, of course, important not to suppress the cough too much, and consequently cough mixtures containing codein or other narcotics are to be avoided except for the purpose of quieting the cough enough to allow the patient to rest. A new form of medicinal therapy has recently been proposed by Nammack and Tiber (35), who had encouraging results from intravenous injection of a guaiacol preparation combined with sodium iodide. Their series of 20 cases is too small to allow any far-reaching conclusions.

2. Arsenicals. Intravenous arsenicals, usually in the form of neoarsphenamine, are often ordered routinely in cases of lung abscess, but most authors are of the opinion that arsenicals are worthless unless numerous spirochetes and fusiform bacilli are found in the sputum. Even in these cases, though the breath and sputum usually become less foul, the course of the abscess itself is often little affected. Cutler and Gross (14), King and Lord (25) and many others have found the results following the use of arsenicals to be, on the whole, disappointing. However, there is a small percentage of pulmonary infections in which these organisms are found in great abundance in the sputum. Such abscesses are usually of short duration and not well localized, and often clear up rapidly under treatment with intravenous arsenicals, in such a way as to make it appear that the Vincent's organisms were the ones primarily responsible. These cases of pulmonary fusospirochetal infections are not commonly found in the series of lung abscesses reported by most authors, though Kline and Berger (27), Pilot and Davis (40), D. T. Smith (43), and others stress their fundamental importance and the efficacy of arsenical therapy. We have had a small number of cases which seemed to derive marked and rapid benefit from this form of treatment, but as a routine measure it has been disappointing. Various preparations of bismuth may also be used in conjunction with the neoarsphenamine.

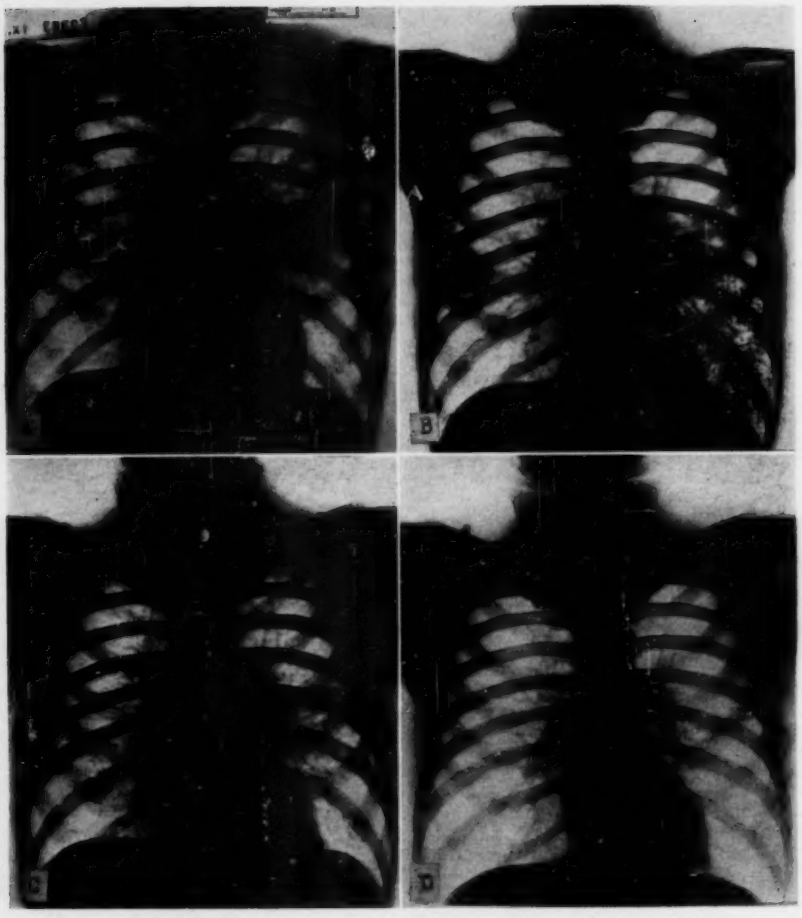

FIG. 3. AN ABSCESS OF LESS THAN 2 MONTHS' DURATION, TREATED CONSERVATIVELY, WITH SLOW BUT EVENTUAL RECOVERY

J. C., male, colored, aged 46 years. Duration of abscess prior to admission: 1½ months. Onset: One day after laparotomy for stab wound in the abdomen; presumably post-operative atelectasis with pneumonia. Marked dental caries and pyorrhea. Many fuso-spirochetal organisms in the sputum. Treatment: Postural drainage, neoarsphenamine 8 times, bismuth 11 times. Result: Complete cure.

Photographs: (a) September 8, 1937: Large lesion with cavity on the left, smaller one on the right. (b) September 17, 1937: After lipiodol which has not entered the cavity. (c) December 4, 1937: Marked clearing. (d) August 8, 1938: Lungs clear.

3. Other drugs. An additional drug which certainly should prove valuable in the rare cases due to beta hemolytic streptococci is *sulfanilamide.* It was employed in one of our patients and seemed to have a beneficial effect. In the cases in which the abscess directly follows pneumococcus pneumonia (whether lobar or bronchopneumonia) and in which the suppuration is presumably due, at least in part, to the pneumococcus, *sulfapyridine* should be tried early. However, it is questionable whether this drug will have any effect on the lung abscess, once it is fully developed. So far neither of these drugs has been found effectual against anaerobic bacteria, and inasmuch as these organisms apparently play the most important rôle in lung abscess, at least in the chronic cases, there is every reason to believe that they will not add much to the therapy except in rare cases. *Iron* in various preparations is useful in combating the anaemia that so often is present. *Vitamin* preparations should be used for those patients who show any signs of vitamin deficiency, or who are not able to take a diet containing adequate amounts of vitamins. There is considerable experimental evidence indicating the importance of adequate amounts of vitamin A in the diet in maintaining the integrity of the epithelial lining of various structures, including the bronchi. Lack of this vitamin may lower the resistance of such tissues to bacterial invasion. It is also necessary to see that the diet contains an abundance of vitamin C, since the stores of this vitamin are often depleted in infections of all sorts, and many lung abscesses run a septic course for weeks or months.

4. Postural drainage. If drainage of the abscess can be accomplished through the bronchus, every effort should be bent toward promoting this result, before considering surgical intervention. Many patients can be started at once on the road to recovery by means of postural drainage. Knowing the position of the abscess and the direction of the bronchi draining it, the proper posture for the most adequate drainage can, as a rule, be easily determined. However, the use of this posture should not be persisted in if the drainage is not good, for a patient often finds by experimentation which position produces the best results. The simplest method is to have the patient lie over the side of, or across the bed with his hands on the floor or on a low chair and with the trunk turned so that the affected

side of the lung is uppermost. In many cases various positions will have to be tried. The patient should be instructed not to cough hard at any time but to allow drainage to take place mainly by gravity, with just enough cough to help keep the passages clear. A patient is often wrongly advised to cough vigorously during this period of drainage; in addition to the obvious dangers to the old or debilitated, there is the risk of spreading the pulmonary infection, for during a hard cough secretions in the smaller bronchi may be forced backward into the bronchioles and alveoli, rather than outward toward the trachea. This is the probable cause of the high fever and pneumonic spread of the infection that is often seen in patients with lung abscess or bronchiectasis who are receiving vigorous postural drainage. Consequently, when the drainage by this means is not satisfactory, or when the patient cannot refrain from severe paroxysms of coughing, it should be discontinued. The scheme ordinarily followed is to have the postural drainage carried out for 5 minutes twice a day, gradually increasing it to 10 or 15 minutes two or three times a day. However, this intermittent type of drainage is certainly far from ideal, for during the rest of the day and especially at night when the cough reflex is depressed there is cessation of drainage, and possible spread of the infection to other parts of the lungs following the course of gravity. This problem was considered in detail by Nelson (36) who pointed out that the natural methods of emptying the bronchi, namely ciliary action and cough, are extremely inefficient in these cases, since the former can only take care of small particles and bacteria, while the cough has a tendency to scatter secretions and pus peripherally. Following a careful anatomical study of the bronchial tree, he gave directions as to the most suitable positions for drainage, depending on the exact location of the abscess and the direction of the bronchi in that area. As an aid to maintaining the proper posture, a bed was devised, which could be cranked up in the center, thus allowing more comfortable and prolonged drainage with the patient lying face downward. Morlock (33) reported satisfactory results with this type of bed in cases of pulmonary suppuration. With the patient prone and with the bronchi on the same horizontal plane, the danger of pus spilling over from one bronchus to another is considerably lessened. Bloch and Francis (5) also have devised a postural-drainage

bed which is made to tilt in various positions, while the patient's head extends beyond the end of the bed and over a receptacle for the sputum.

While we do not believe that it is possible in the majority of cases to localize the lesions as precisely as was described by Nelson (36), this should be attempted as carefully as possible by means of X-rays, bronchoscopy, etc., and a plan of drainage outlined accordingly. The drainage should be as nearly a continuous procedure as can be arranged without too much discomfort to the patient. A special apparatus is probably not necessary. In the case of abscesses in the lower lobes, the foot of the bed should be gradually elevated until the patient is accustomed to having it up about 20 inches. In dealing with abscesses in the upper half of the lungs various positions should be tried, but the scheme which is likely to produce the best results is to have the patient in Fowler's position during most of the day and night, lowering the head and trunk at intervals during the day.

5. *Phrenic paralysis.* Although a few authors have found this measure worth trying in occasional cases, the majority mention it only to condemn it as useless. We agree with this attitude, not only on the basis of the few cases in our series in which it was certainly of no help, but we also feel that diaphragmatic paralysis is not without danger in patients with pulmonary suppuration. With a marked rise of the diaphragm the bronchi may be kinked enough to interfere with drainage, and the expulsive force of the cough is less effective when the diaphragm is paralyzed. These changes in pulmonary mechanics may lead to spread of the infection. Because of these dangers and because it is difficult to pick the rare appropriate case, it seems that this method of treatment should be employed only in very exceptional instances.

6. *Pneumothorax.* This form of therapy has been given extensive trial. We believe, on the basis of various reports and our own experience that it is often dangerous, and that it should be employed in only a small proportion of cases. Allen and Blackman (3) believe that its use is not justifiable, on the basis of 26 cases in which they employed it. King and Lord (25) concluded that the results obtained were not only disappointing, but that the procedure entailed the serious risk of inducing empyema. Cutler and Gross (14) reach a similar conclusion.

Miller and Lambert (32) mention the same danger, but say that good results may be obtained by inducing pneumothorax in those cases in which the abscess is close to the hilum. Maxwell (31) also believes that the central or hilar abscesses are the only ones likely to benefit from this form of treatment, but he considers the dangers of pneumothorax to outweigh its usefulness. In chronic cases with thick-walled cavities one certainly would not expect to collapse the cavity by this means. Aside from the danger of causing empyema, there is the danger of spreading the infection to other parts of the lung when a large cavity is suddenly emptied into the bronchi. From our own limited experience we are inclined to agree that this measure is useless in the case of most lung abscesses and is dangerous in all, and that it should be tried only for those abscesses of short duration that are close to the hilum and are not responding to other measures.

7. Bronchoscopic drainage. Routine bronchoscopy is a very important step for location of the abscess, for detecting carcinoma when possible, and for locating and removing foreign bodies. Many times it may promote better bronchial drainage by clearing viscid secretions from the bronchi and by shrinking swollen mucous membranes, thus opening the passages. Likewise, by this means it is possible to remove, by cauterization, granulation tissues that are obstructing the bronchus. Repeated drainage by this method is rarely necessary, however, since equally good results can usually be obtained by postural drainage, with much less discomfort. Many authors believe that bronchoscopy is useful for diagnosis only, but Flick *et al.* (18) maintain that it should be used not only as a diagnostic measure but also for treatment of most cases, especially those not responding quickly to routine medical treatment. Alexander (1) is one of those who feel that much can be done bronchoscopically to improve drainage by removing obstructing granulations. Chevalier Jackson (23) recommends its use especially in abscesses close to the hilum. It certainly should be tried, particularly in the early acute abscesses. If drainage is improved and the patient tolerates the procedure well and shows general improvement, it may be well to repeat it. However, long periods of bronchoscopic drainage are very seldom indicated.

8. Transfusion. This is helpful in many cases and should not be withheld until the patient is very anaemic from loss of blood. Used early, even if there is only slight anaemia, this is an additional proce-

dure in the medical régime that often seems to be of marked benefit. It is also a necessary step in most cases in preparation for surgery or immediately after operation.

C. Surgical treatment

The most satisfactory surgical treatment in most everyone's hands has proved to be external drainage of the abscess, done preferably in two stages. Safe surgical drainage requires accurate localization of the abscess, and the determination of its nearest point to the parietal pleura. The first stage of the operation consists in rib resection with exposure of a reasonably wide surface of pleura. The wound is then packed for at least forty-eight hours. At the end of this time, using the actual cautery, the abscess cavity is opened widely and the cavity packed with gauze. There are a few cases in which the parietal and visceral pleurae are adherent when first exposed, and, of course, it is then possible to carry out the drainage in one stage. However, if there is the slightest question as to whether the pleural surfaces are adherent, the two-stage procedure is utilized. As has been pointed out many times by others, there is a great temptation to put a needle into the chest in order to confirm the localization of the abscess before operation. This is a dangerous procedure, and it should not be done.

There has been considerable controversy regarding the time when surgical drainage of an abscess should be instituted. There are no definite rules that can be applied to all cases: one should be guided by the course of the disease.

Miller and Lambert (32) feel that operation will be necessary in approximately 40 to 50 per cent of cases. However, they warn of the dangers of operation in the acute stages before the abscess is walled-off.

Cutler and Gross (14) have advised six weeks' medical treatment, followed by drainage in one or two stages, depending on whether or not the pleural surfaces are adherent. Incision of the abscess is made with the endothermy knife if the lesion is peripherally located, and with the actual cautery if it is of the central type. They feel that this lessens the danger of hemorrhage and air embolism. They also recommend vertical incisions rather than those parallel to the ribs: they believe that the former afford better drainage because the wound stays open longer.

Brunn and Goldman (6) use the two-stage operation even when the

pleural surfaces are definitely adherent. They believe that delay leads to poor surgical results by allowing multiple abscess cavities to form. Earlier in the disease there are usually only single cavities which can be drained more successfully.

Lobectomy has been carried out successfully in the treatment by a number of surgeons, but this procedure is only applicable to a few selected cases. The results as a whole have been poor; therefore the procedure is not recommended.

We believe, after studying our own cases and reviewing the reports of others, that Neuhof and Touroff (37) are right when they make a plea for early operation in lung abscess. Their recent report of forty-five cases with only three deaths lends strong support to their argument.

Alexander (1) has also stated that he now believes that when most lung abscesses have been given proper and complete medical treatment for a period of about three weeks without showing a good response, surgical drainage should be seriously considered.

We feel that if the lesion is not showing definite improvement at the end of three to five weeks, surgical intervention is usually indicated. In general, the peripherally located abscesses are less apt to clear up under medical management and are more amenable to surgery. Therefore, operation should be resorted to earlier in the treatment of this type of abscess than in the centrally situated ones, in which the medical régime is more apt to prove effective and surgery more hazardous.

Post-operative Complications. The most common serious complications following operations are: (1) The spilling over of infected material from diseased into healthy areas, causing a dissemination of the infection or immediate bronchial obstruction. The importance of this as a causative factor in many of the post-operative deaths was stressed by Faulkner (16); it emphasizes the importance of the most gentle operative procedures. (2) Pyo-pneumothorax—usually a very virulent anaerobic infection of the pleural cavity, which makes the patient desperately ill, and the prognosis grave. This complication necessitates immediate operation, preferably some form of closed pleural drainage. Pleural irrigations with zinc peroxide solutions may prove useful; they were used successfully in one of our cases. In our group

there were 9 instances of pyo-pneumothorax with 5 deaths. (3) Hemorrhage—occurring most often after operation on chronic abscesses where the thick fibrotic walls do not collapse after drainage of the abscess. It is less apt to occur if the cautery is employed at operation; but when it occurs it calls for repeated cauterization, packing and transfusions. (4) Persistent bronchial fistula, which may need secondary operations for closure, as was the case in one of our patients.

RESULTS

It is very difficult to evaluate the results of the various types of therapy in a condition such as this, into which so many variable fac-

TABLE VI

Results of treatment

Approximate figures for combined medical and surgical treatment

AUTHORS	NUMBER OF CASES	WELL OR IMPROVED	MORTALITY
		per cent	*per cent*
Allen and Blackman	94	68	30
King and Lord	210	58	37
Cutler and Gross*	85	58	38
Warner	98	53	47
Neuhof and Touroff	45	88	4
Lueth and Sutton	101	50.5	39
Fisher and Finney (present series)	88	57	41

* Total in series was 90; 5 were not followed.

tors enter. We know the prognosis is greatly influenced by the duration of the illness, by the general condition of the patient, by the etiological factors, the bacterial flora, the position of the abscess, *etc.* Moreover it is not reasonable to speak of the medical, as opposed to the surgical mortality, when we know that the surgical cases usually are medical failures.

Table VI gives a general impression of the prognosis of this condition. The close correspondence of most of the figures is noteworthy. It is interesting that Allen and Blackman (3) were able to find reports in the literature of 2,114 patients with lung abscess of which approximately 50 per cent were treated conservatively and 50 per cent

surgically, with a mortality of 34.4 per cent for those treated conservatively and 34.2 per cent for the others.

The results in our series are shown in Table VII. The patients referred to as "well" have shown definite evidence by X-ray and symptoms of complete resolution of the abscess and many have had long

TABLE VII

Summary of results

Well	32	36.3%
Improved	18	20.4%
Unimproved	2	2.3%
Died	36	41.0%
	88	100%
Successful results	50	57.0%
Unsuccessful results	38	43.0%
	88	100%

TABLE VIII

Analysis of treatment of 50 patients who either improved or recovered completely

I. Medical treatment—32 cases
   (A) General medical régime—18 cases (postural drainage; high calorie diet; sodium iodide intravenously in 4, staphylococcus toxin in 2, etc.)
   (B) Same as (A) plus neoarsphenamine intravenously—10 cases (bismuth was also used in 3 of these)
   (C) Same as (A) plus two or more bronchoscopic treatments—3 cases
   (D) Pneumothorax—1 case
II. Surgical treatment—18 cases (most of these had a preliminary course of medical treatment)
   (A) Rib resection and drainage—13 cases
   (B) Thoracotomy and drainage of empyema—4 cases
   (C) Pneumothorax followed by rib resection and drainage—1 case

periods of follow-up. Those called "improved" were not entirely free of pulmonary changes by X-ray and usually had some cough, but were able to be about in a satisfactory way. The ones who were "unimproved" were so diagnosed on discharge; they were lost track of and almost surely died.

In Tables VIII and IX we have tabulated the various forms of

therapy employed in the successful and the unsuccessful cases, respectively.

In Table X we have attempted to gather together the more important conditions which seemed to influence the outcome adversely in the 38 unsuccessful results.

TABLE IX

Analysis of treatment of 38 patients who either died or failed to improve

I. No treatment (other than supportive)—7 cases (had fulminating infections and died from a few hours to a few days after admission)
II. Medical treatment—6 cases (routine medical régime; 3 received neoarsphenamine; bronchoscopic treatment in several; 3 refused operation)
III. Surgical treatment—25 cases (12 of these had a preliminary course of medical treatment).
  (A) Rib resection and drainage—14 cases.
  (B) Thoracotomy and drainage of empyema—3 cases
  (C) Extra-pleural pneumolysis (in preparation for pneumonectomy)—3 cases (one of these preceded by pneumothorax)
  (D) Phrenicectomy—2 cases
  (E) Rib resection and drainage, plus phrenicectomy—2 cases
  (F) Pneumothorax followed by ligation of pulmonary artery—1 case

TABLE X

Conditions which probably contributed to poor results in 38 cases

	cases
I. Chronic abscess (6 months–7 years)	15
II. Multiple abscess cavities	11
III. Acute fulminating abscess (often with gangrene, leading to death in a few days to a few weeks)	10
IV. Massive hemoptyses or post-operative hemorrhages	7
V. Development of putrid empyema (pyopneumothorax)	5
VI. Alcoholism of marked degree	6
VII. Patients over fifty-five years of age	10
VIII. Abdominal operation in presence of marked oral sepsis	4
IX. Metastatic abscesses	3
X. Abscess following serious trauma	2

DISCUSSION

We do not feel that we can draw from the material presented in this review, any very definite conclusions as to the many unsettled and controversial aspects of lung abscess. However, from our own series

of patients and from a careful consideration of some of the outstanding reports in the literature, the following statements seem justified.

Our experience serves to reemphasize the importance of respiratory infections such as pneumonia in the *etiology* of one group of cases of lung abscess. In another group of cases surgical operations are the precipitating factors. In still another, quite large group, the precipitating factors are unknown. In the cases following tonsillectomy and other operations on the upper respiratory tract, the evidence favors aspiration of infected material as the most likely cause. It is known that aspiration of material from the nose or throat into the lower bronchial tree may occur during sleep, and is particularly apt to occur during deep anaesthesia and when the cough reflex has been abolished. Moreover, lung abscess can be produced experimentally with the type of material found in human lung abscess, if it is put in a small bronchus and the bronchus is blocked. The weight of evidence favors the view that the primary steps in the formation of the majority of lung abscesses are: first, atelectasis (whether during a respiratory infection or post-operatively), and secondly, aspiration of infected material from the upper respiratory tract. A much smaller proportion are due to infected pulmonary emboli, or sterile emboli followed by aspiration of infected material. Some of the outstanding contributing factors are oral sepsis, alcoholism and malnutrition.

The *bacteriology* of lung abscess is not a subject on which one can write with any finality, since the flora in the material from the abscesses varies so much from case to case. The great importance of anaerobes and facultative anaerobes seems reasonably well established but in many cases aerobic organisms are found to predominate. From our own material the alpha-hemolytic (viridans) streptococcus was very prominent in the sputum; it was at times aerobic and at other times facultatively anaerobic. Anaerobic streptococci were most often found in cultures of material obtained from the abscesses themselves or from the complicating empyemata. Anaerobic Gram-negative bacilli were also frequently found in the abscess or empyema fluid, but were not often isolated. We found fusiform bacilli and spirochetes in significant numbers in about one quarter of our cases, but except in a few patients who responded well to neoarsphenamine therapy, we could not convince ourselves that these organisms played

more than a secondary rôle. Many other aerobic and anaerobic bacteria were cultivated and in most lesions a symbiosis of several varieties was encountered.

The differential *diagnosis* of lung abscess has not been dealt with in any detail in this report, but the most important conditions to be considered are tuberculosis and carcinoma of the lung. Repeated sputum tests for tubercle bacilli by concentration and culture, the history, the character of the sputum and the X-ray findings will usually rule out tuberculosis. Carcinoma of the lung or bronchus is often much harder to differentiate because it is very common for it to become secondarily infected and present the symptoms of lung abscess. An X-ray shadow radiating outward from the hilum and not well demarcated, atelectasis of a lobe, the finding of bloody pleural fluid are all signs favoring the diagnosis of carcinoma. Bronchoscopic examination will often reveal the bronchial growth from which a specimen can be taken for biopsy, and at other times the bronchus may be seen to be compressed by an extrabronchial mass. In many cases, however, the diagnosis cannot be made until operation and even then the thick fibrous wall of a chronic abscess may simulate that of an infected carcinoma very closely, so that in doubtful cases the issue can only be settled by biopsy. Bronchiectasis with secondary abscess is best differentiated by the long history and by lipiodol study. A subacute or chronic empyema situated either in an interlobar fissure or at the base of the lung is another possibility to keep in mind.

We have discussed in some detail the *treatment* recommended by various authors, as well as that employed in our cases. We should like especially to stress the importance of *prophylaxis* which concerns itself mainly with the eradication of infections in the nose, throat and mouth, especially before operation. The type and depth of anesthesia and the position of the patient during the operation are important considerations. Other prophylactic measures which have been stressed are aspiration of secretions from the trachea during and after operation, and inhalations of a mixture of CO_2 and O_2 post-operatively in patients who are not aerating their lung bases well, which is most likely to happen after abdominal operations.

We believe that a higher percentage of cures will result if the medical management is begun early and carried out with careful attention to

details. The general measures useful in combating any prolonged infection apply here, and the patient should have a carefully supervised course of postural drainage. This drainage should be made as nearly continuous as is practical. Arsenicals intravenously are not useful as a routine, but when the sputum contains many spirochetes this form of treatment at times yields dramatic results. Bronchoscopy is employed routinely for diagnosis, for localization of the abscess, at times for improving drainage by cauterizing obstructing granulations or sucking out plugs of mucus and pus, for removal of foreign bodies, *etc.* Repeated bronchoscopic treatments are usually not indicated. Pneumothorax is useful only in an occasional case when the lesion is close to the hilum.

As for the *surgical treatment* no rigid rules can be laid down to govern when or how it is to be done. Each individual case presents its own problems. The trend now is toward earlier operation, but it should not be so early that the patient is not given a good chance for recovery under a medical régime. The aim of surgical treatment should be to provide adequate drainage without contaminating the pleural cavity. This can usually be accomplished safely and efficiently by a two-stage operation, using the actual cautery for incision into the abscess.

Among the conditions which seem to affect the prognosis unfavorably, a long duration of the abscess previous to therapy is an item of great significance. In the *chronic abscesses* the chief barriers to successful treatment are (a) thick, rigid, fibrous walls which prevent collapse of the cavities, (b) eroded blood vessels with recurrent hemorrhages, as long as the cavities remain open, (c) intra-pulmonary spread or metastatic abscess formation, and (d) the unfavorable effect of the chronic infection upon the patient's general condition. Any sort of therapy is, of course, made more difficult when *multiple abscesses* are present. *Acute fulminating abscesses* usually lead to death in a few days to a few weeks in spite of treatment. The rapid spread of these infections may be due to the presence of organisms of greater virulence, or to a lowered resistance of the tissues. *Putrid empyema* or *pyopneumothorax* was present in 9 of our patients; at the present time it seems that some form of closed drainage offers the best means of dealing with these complications.

A history of *chronic alcoholism* certainly seems to make the prog-

nosis much worse, since the total number of our patients who gave a history of marked chronic alcoholism was 6 and all of these died. It was mentioned earlier that alcoholism may be a contributing factor in the development of lung abscess. Alcoholic patients are apt to suffer from vitamin and other nutritional deficiencies which tend to lower the resistance of the tissues to bacterial infections. Of interest in this connection is the recent work of Pickrell (39) who showed that the lungs and other tissues of rabbits during alcoholic intoxication had lost their resistance to invasion by the pneumococcus, owing apparently to failure of the leucocytes to emigrate from the capillaries in the normal way.

The danger of carrying out operations of any type in the presence of infections in the mouth was demonstrated by our experience in four fatal cases in each of which a pulmonary abscess developed after an abdominal operation upon a patient with marked oral sepsis.

SUMMARY

(1) An analysis is presented of 88 cases of lung abscess; of these 57 per cent are well or improved and 41 per cent dead.

(2) On the basis of experimental work and clinical observation, atelectasis and aspiration seem to be of fundamental importance in the pathogenesis of the majority. Embolism accounts for a much smaller number.

(3) Respiratory infections contributed the largest number of cases (49 per cent) in our series, while 28 per cent were post-operative.

(4) The bacterial flora of the abscess itself is predominantly anaerobic, with anaerobic streptococci and Gram-negative bacilli the organisms most frequently present in significant numbers.

(5) Various prophylactic measures have been outlined. Careful attention to these details should markedly lower the incidence, especially of the post-operative group.

(6) The details of a carefully planned program of medical therapy should be strictly enforced, with particular emphasis on efficient postural drainage. Under this régime a high percentage of the early cases will be cured.

(7) Surgical treatment is often withheld too long. We feel that this should be considered when a patient fails to show improvement

after approximately three to five weeks of conservative treatment, though the interval will vary from case to case.

(8) The various surgical measures are discussed. The more conservative approach has yielded the most satisfactory results.

(9) Some of the conditions which were found to influence the outcome adversely, in addition to the patient's age and general condition, were the chronicity of the abscess, pre- or post-operative hemorrhage and chronic alcoholism.

(10) It is our feeling that more prompt, and better coordinated treatment will greatly reduce the mortality in lung abscess.

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THE EFFECT OF TESTOSTERONE PROPIONATE ON THE DEVELOPMENT OF TUBERCULOSIS IN THE IMMATURE MALE GUINEA PIG

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The accumulated observations over the past few years have considerably broadened the significance of the sex hormones in the animal economy. It is well known that they may exhibit profound physiological effects in addition to those first observed on the reproductive tract. It is a plausible scheme then to seek the influence of these substances in any circumstances in which differences in sex, in the state of sexual maturity, or in sexual function (e.g. pregnancy) are known to be important. Alterations in the susceptibility to certain infections associated with sexual differences have already led to some fruitful investigations.

Gonorrheal vaginitis in children has always been a serious problem because of its high infectiousness and stubborn persistence. In women it is usually so transitory as to be of negligible importance. The discovery by Lewis (1) of the rapid, favorable effect of estrogens on this infection was based largely on the recognition of the anatomical effect that this hormone had on the vagina.

Gingival infections like those associated with pregnancy in women have been observed by Ziskin and associates in castrate monkeys (2) and in women (3) receiving pregnancy urine extracts. The character of the epithelium was altered and the occurrence of these infections prevented in monkeys and women receiving estrogenic preparations.

The lesser extent of vaccinial lesions in female rabbits, especially pregnant ones, was significant in a large colony of rabbits vaccinated by Rosahn, Hu and Pearce (4). Sprunt, McDearman, and Raper (5) demonstrated that a preceding treatment with large doses of estrogenic hormone reduced the number of successful inoculations of vaccinia virus. Treatment with gonadotropic hormone had an equivocal effect, however.

Male rats have a measurably lower resistance to cysticercus infestation than

[1] National Research Council Fellow.

the female. Campbell (6) found that although preparatory injections of theelin caused only a questionable increase in the males' resistance, as measured by number of cysts in the liver seven weeks after infection, the preparatory administration of testosterone propionate caused a very significant decrease in the resistance of the female to a comparable level with that of the male.

Less convincing, but still very suggestive results have been obtained in the study of experimental poliomyelitis infections. Common clinical impressions on the incidence of poliomyelitis in pregnancy led Jungeblut and Engle (7) to discover that the blood serum in pregnancy had a definitely higher neutralizing power than normal serum. They found great fluctuations in the neutralizing effect of an individual's serum also but this could not be related to the phase of the menstrual cycle. Nevertheless in a large number of experimental observations they found complete protection from intracerebral inoculation of the virus in a few monkeys after prolonged pretreatment with a gonadotropic preparation (8). This mode of infection invariably succeeded in a large number of controls. Subsequently Aycock (9) reported some evidence of protection against intranasal infection in a small number of monkeys.

The basis for an interest in the possible effect of sexual maturity in tuberculous infection can be readily appreciated by reference to the recent lecture by Rich (10) on the "age-determined" factors which play such a conspicuous role in the development of human tuberculosis. The mortality rate among infected individuals shows striking differences at different periods of life. Following the very high case fatality rate of infancy there comes a period from five years to puberty when the benignity of tuberculous infection is truly remarkable. This period of grace is followed in turn by a mounting rate of fatality in adolescent years and never again does it return to the low level of childhood. At about the time of puberty certain sex differences in the mortality per capita begin to appear. It rises much more rapidly in the female to a peak in the middle of the childbearing period. In the male the rise is more gradual but at about the age of forty years it passes and thereafter greatly exceeds that of the female. Finally there is a further rise in both sexes in very old age.

It is obvious that there may be many factors that operate to produce these several variations in the severity of tuberculous infection according to the age and sex of the individual and these have been discussed at length. No simple explanation has been given, however, for the difference in the prognosis of the disease between the childhood and adolescent periods. It seems unlikely that this can be

explained entirely on the delayed fatal outcome of cases infected in an earlier decade. Clinically the disease in young children shows a tendency to heal rather than progress and become chronic. It may be merely a coincidence that this accentuation in the gravity of the disease is synchronous with the development of sexual maturity. However, the suggestiveness of their association is too plain to ignore.

A recognition of some of these facts led early workers to seek the effect of sex alterations on experimental tuberculosis. The available experimental method in the earlier work was castration. In brief summary it may be fairly said that the early work (11, 12, 13, 14) was always marred by the use of too few animals and the failure of application of any good objective measure of the resistance of the animal (length of life after infection was the usual criterion). Although Bourgeois and Boquet (15, 16) have continued to observe longer survival in castrate animals infected with tuberculosis, they have not recorded any estimate of the extent of generalization after identical periods of infection. This has been done by van Bokkum (17) and he has found no difference in the extent of infection nor in survival time in males and females castrated five months before infection.

In an attempt to learn the effect of hormones administered during the course of the infection, Addessi (18) injected infected rabbits with pregnancy urine and found that they died earlier than their controls. It is needless to comment on this. A number of workers (15, 19, 20, 21) have subsequently used more refined preparations of estrogens without finding any effect on the infection. This has been particularly thoroughly studied by Gray and Brack (21) with large numbers of female guinea pigs and in carefully controlled experiments. No effect of large doses of crystalline hormone was found on the character of the infection in either first-infected or previously immunized animals.

Some of the foregoing investigations (16, 18, 19) tested the effect of gonadotropic hormones as well without obtaining any convincing results. Standard gonadotropic preparations have since been employed in rabbit and guinea pig tuberculosis by Steinbach and Klein (22) and Gray and Brack (23) with discordant results. Bourgeois and Boquet (15) found no effect of "corpus luteum" on the survival period of infected animals. Greene and Morgan (24) have since reported identical degrees of spread of the infection in animals treated with crystalline progesterone and their controls. Although the treated animals lost more weight, the tuberculin tests in the two groups were alike two weeks after infection.

We have found but one report of the use of an androgen in experimental tuberculosis. Bourgeois and Boquet (16) reported that 5 male guinea pigs, given 5 mg. crystalline "male hormone" weekly lived 8 months after infection in contrast to 5 females and 5 castrate males similarly treated, which died in the 5th month. Twenty male and female controls lived from 4 to 6 months, while 10 female castrates lived 6 months and 10 male castrates lived 8 months. Male hormone, therefore, seemed to have the same effect as castration in prolonging the life of

the male guinea pig infected with tuberculosis. These authors made no study of the dissemination of the tuberculous lesions.

There is little information in the literature on changes in sex hormone secretion during sexual maturation in the human. Oesting and Webster (25) have reported the most numerous observations on urinary excretion of sex hormones during childhood. The limitations with which we are beset in interpreting the significance of hormone excretion in terms of gonadal activity (26) do not detract from the importance of these data. Their searches never revealed any estrogenic hormone excretion in boys or girls until the age of puberty was approached. Some androgenic activity was found, however, in all the children's urine even down to the youngest, aged three years. It also appears from their data that a definite and significant rise in the quantity of androgen excreted begins about the 6th year and there is a steady rise thereafter. It seems unjustified to neglect the androgens in an attempt to discover the effect of sexual alterations on experimental tuberculosis.

METHOD

Thirty-two young male[2] guinea-pigs were obtained at ages 3, 4, or 5 days together with the nursing mothers. These were segregated, as littermates when possible, into groups of four young and a mother. Care was taken in every instance to include pigs of as nearly the same weight as possible in the same cage. They were given abundant feedings of cabbage, grass, lettuce, celery tops, carrots, hay, oats, oatmeal, and pellets of Purina Chow. Water was given after the first week. The mothers were removed from the cages when the average age of the young was 28 days.

The young were numbered serially and the day following their segregation each odd numbered animal received a subcutaneous implantation of two pellets of crystalline testosterone proprionate (Ciba) beneath the skin of the back. The combined weights of each pair of pellets average ± 13.6 mg. In this way there were two treated and two untreated animals in each cage and the average body weights of the treated and untreated groups were almost identical.

Three days later, when the average age was eight days, every young animal was infected by subcutaneous inoculation in the left groin of 0.1 cc. volume of a uniform suspension of virulent tubercle bacilli. These organisms were supplied

[2] One female, animal no. 22, inadvertently included in the control group, was allowed to remain and was treated like the others, but has been omitted from the data.

to us by Dr. R. H. Follis, Jr. from a culture of human sputum strain grown on Petroff's medium. A uniform suspension was made by lightly grinding a pure colony of the bacilli in a mortar with gradual addition of sterile saline solution and brief centrifugation to throw out the clumps. The suspension utilized showed an average of 5 bacilli per oil immersion field and no clumps when a drop about 1 cm. in diameter was dried on a slide and stained with Ziehl-Neelson's carbol fuchsin.

Weight records were kept of each animal at about weekly intervals after infection. Fourteen days after infection a tuberculin test was carried out on each animal. This consisted in the intradermal inoculation of 5 mg. of Old Tuberculin (U.S.P.H.S. Stock) in 0.1 cc. saline into the shaved skin of the side. A similar quantity of control solution was injected. The reactions were read after 24 and 48 hrs. Two weeks later, or four weeks after infection, another tuberculin test was done. On account of the poor reactions to the first test, 10 mg. quantities of the same batch of Old Tuberculin were used in the second test. The animals were subjected to no other manipulations.

On the forty-second day after infection all the animals were killed with ether and immediately autopsied. The spleens, livers, lungs with tracheas and lymph nodes, were removed and fixed in 10 per cent formalin. The testes, prostates, and seminal vesicles were removed and fixed in Bouin's solution for 24 hrs. and then weighed. The carcasses and remaining organs, after the cranial cavities had been opened, were fixed in 10 per cent formalin for 24 hrs. and then the adrenals and hypophyses were dissected out and weighed. Histological sections were made of all the organs mentioned. Carbol fuchsin was used to identify the tubercle bacillus in the lesions. Each pellet of testosterone propionate was recovered, cleaned, and weighed in order to determine the amount absorbed during the experimental period.

RESULTS

Course of the Disease. None of the animals died during the course of the experiment and none showed signs of severe illness. They all continued to gain weight at variable rates. Figure 1 shows that the growth curves of the two groups had almost identically the same slope. The hormone treated group showed an average gain of 200 per cent of the initial body weight at the time of infection, while the control group showed an average gain of 216 per cent of the initial weight. The slightly lower final average weight of the treated group is due to the occurrence of the four lightest animals in this group. This has no apparent relation to the course of the infection, however, since examination of the data reveals that these four animals had an average extent of tuberculous lesions practically identical with that of the entire treated group.

Primary Lesion. All the animals developed a small hard nodule at the site of inoculation. Within 4 weeks after infection three animals in each group had small ulcerations over these nodules. The final average degree of tuberculosis in the animals which developed ulcerated primary lesions was the same as in the group as a whole. The average severity of tuberculin reactions of these animals also showed no significant deviation from that of the group. The average weight of these animals at the time their ulcerations appeared was 267 gms. as compared to 281 gms. for the entire series at the same time.

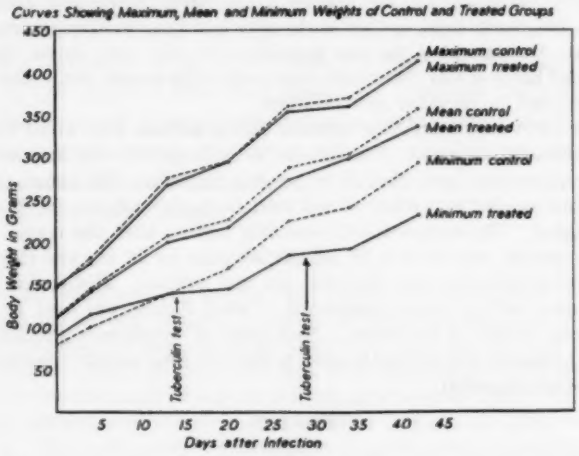

FIG. 1

Allergy. The reactions to the first intradermal test (5 mg. Old Tuberculin) carried out on the fourteenth day of infection, were so feeble as to be negligible. The animals at this time were of an average age of 22 days. The second test, conducted on the twenty-ninth day of infection when the animals were 37 days old, was a 10 mg. quantity of Old Tuberculin. The reactions to this test were more severe. Table 1 reveals no very convincing difference between the average reaction of the two groups. It is apparent that in both the treated and control groups the more severe reactors tended to be the larger animals. Although the table does not show it, the ultimate

extent of tuberculosis found at autopsy two weeks later bore no relation to the degree of reactivity to this test.

TABLE 1

Reaction to Old Tuberculin (10 mg. intradermally)

TUBERCULIN REACTION	TREATED		CONTROL	
	Number animals	Average weight	Number animals	Average weight
		gm.		gm.
+	9	259	5	267
++	5	287	10	299
+++	2	309	0	

Animals aged 36–38 days; infected 29 days.

TABLE 2

Estimate of Extent of Tuberculosis on Gross and Microscopic Examination

HORMONE TREATED						CONTROL					
Animal number	Spleen	Bronchial nodes	Lung	Liver	Total	Animal number	Spleen	Bronchial nodes	Lung	Liver	Total
1	1+	0	0	1+	0.5+	2	2+	0	1+	3+	1.5+
3	4	2+	3+	2	2.8	4	2	0	1	3	1.5
5	3	1	1	2	1.8	6	2	2+	2	1	1.8
7	1	0	2	0	0.8	8	3	2	2	2	2.3
9	3	4	3	3	3.3	10	3	4	3	3	3.3
11	4	1	1	1	1.8	12	1	1	1	1	1.0
13	4	0	3	3	2.5	14	4	3	1	1	2.3
15	3	0	1	1	1.3	16	1	1	1	1	1.0
17	3	0	0	0	0.8	18	2	1	0	0	0.8
19	3	1	1	1	1.5	20	3	4	1	1	2.3
21	3	0	0	1	1.0	22					
23	3	2	0	2	1.8	24	2	1	2	0	1.3
25	2	1	1	1	1.3	26	2	1	1	0	1.0
27	3	1	0	2	1.5	28	3	4	1	2	2.5
29	1	1	0	1	0.8	30	3	3	1	3	2.5
31	4	4	4	4	4.0	32	4	4	1	2	2.8
Average	2.8	1.1	1.3	1.6	1.7+		2.5	2.0	1.3	1.5	1.8+

Distribution of Lesions. In addition to the local lesion in the groin and very large retroperitoneal lymph nodes, there was some degree of generalization of the infection in every animal. The relative extent of tuberculosis in each animal is recorded in Table 2. In

the spleens and bronchial lymph nodes the estimates given are based on the size of the organ and number of macroscopic tubercles, confirmed by microscopic sections. In the lungs and livers only occasional animals had any grossly visible tubercles so that the estimates are based on the count of tubercles in a single large transverse section of the liver and one coronal section through each lung. The serous cavities were never involved and no gross tubercles were seen in other organs. The table shows no significant difference in the extent of the disease in the two groups.

Microscopically no difference could be appreciated between the amount of caseation of the lesions in the two groups. None of the animals had any pneumonia in association with the pulmonary tubercles.

TABLE 3

Relative Mean Organ Weights (mg. per 100 gm. body weight)

	TESTES	PROSTATE	SEMINAL VESICLES	ADRENALS	HYPOPHYSIS
Treated.....	47	189	237	31.3	2.8
Control.....	320	164	190	30.5	2.8

Tissues fixed 24 hours before weighing.

Absorption of Drug. The recovered pellets of testosterone propionate were weighed and the quantity of drug absorbed calculated. The total quantity absorbed in the forty-five days of implantation averaged 7.2 mg. (±17 per cent). The variations were probably dependent both on the differences in original pellet weights and on some unavoidable slight fragmentation caused by inserting them through a needle with a stilette. Other unknown factors may have played a rôle. Body weight apparently was not one of these since the average absorption in the eight heaviest animals was identical with that of the eight lightest animals.

Endocrine and Accessory Sex Organs. In Table 3 the pertinent organ weights, expressed in relation to body weight, of the two groups are given. The prostates and seminal vesicles of the hormone-treated group definitely average higher than those of the control group, although this difference is not very marked. The testes of the treated

group were invariably much smaller than those of the control animals. This striking difference is well shown in the scatter diagram (figure 2). There was no significant difference in the weights of the adrenals and hypophyses of the two groups.

The histological differences in the testes are just as uniform and as marked as were their differences in weight. In figure 3 representative fields of a testis from each group are compared with that from a normal immature guinea-pig of a younger age. The testis of the hormone *treated* animal is essentially like that of the much younger normal animal. The small tubules are lined by an almost single

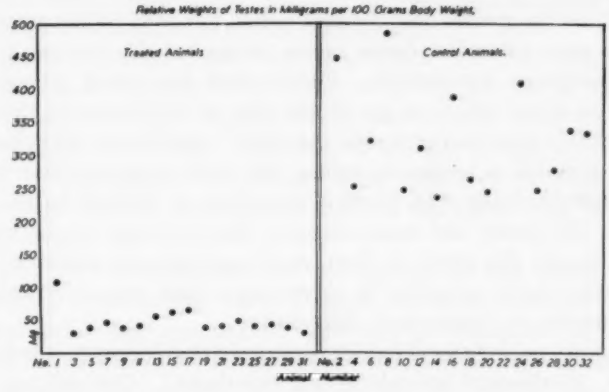

FIG. 2

layer of cells which are largely Sertoli cells and resting spermatogonia. A rare primary spermatocyte division occurs. The interstitial tissue is composed of small spindle-shaped, connective tissue-like cells. The testicular tubules of the *control* animals are much larger and their lumina practically filled with dividing germinal cells. There are large numbers of primary spermatocyte divisions and even numerous sperm heads, although no mature sperm are found. There are also numerous nests of plump, eosinophilic, interstitial cells.

The epithelium of prostates and seminal vesicles is tall and secretory in both groups alike. No differences are recognized in the structure of the adrenals. No fine cytological study of the hypophyses has

been made but, when stained with an aniline blue-eosin mixture, the proportions of eosinophils and chromophobe cells seem to be alike in the two groups and basophil cells are few in both.

DISCUSSION

The studies of the relative resistance of immature and mature guinea pigs to tuberculosis have been inconclusive. Krause (27) observed less dissemination as well as smaller local lesions in young guinea pigs inoculated *intracutaneously* than in older ones given the same quantity of bacilli. After *subcutaneous* inoculation with identical quantities of bacilli, the younger pigs had the more extensive disease. He believed that the greater dose of bacilli per unit of body weight accounted for the more extensive disease in the young but this was not proven by appropriate experiments. Furthermore his young guinea pigs were five to six weeks of age at the time of infection and therefore were closely approaching sexual maturity. Smithburn (28) observed longer survival in immature guinea pigs than in mature ones inoculated *intracerebrally* with identical quantities of tubercle bacilli. Although the young had more extensive dissemination of the lesions, he attributed this solely to their longer survival and concluded that they were more resistant to tuberculosis than the older animals. This might be interpreted differently.

We make no pretense at having produced a true "premature puberty" in the experimental animals in our experiment. Our rationale has simply been to learn the effect on the course of experimental tuberculosis of a type of hormone, the secretion of which is at least quantitatively influenced by sexual maturity in the human (25). In order to accentuate the effect of our treatment we have chosen an immature animal in which the natural occurrence of this hormone may be presumed to be minimal. The use of the male animal is a further simplification. Our entire period of experimentation was designed to fall within the period of sexual immaturity of this animal's life (29). The animals were seven weeks old at the end of the experiment and none of them showed mature spermatozoa in the testes or epididymes at this time, although several of them had very slight tuberculous infections.

The administration of the crystalline steroid compounds in the

form of small, compressed pellets, implanted subcutaneously, has been found to be very useful in the hands of several investigators in the past two years. It is common experience that a slow steady absorption of the drug administered in this way gives a much greater effect than the same quantity administered in one or two daily injections in oily solution. Another advantage of this mode of treatment in the present experiment is that the animals are subjected to only one injection at the beginning of the experiment and the effects of daily handling and trauma are dispensed with. Follis (30) has shown the effect that such daily manipulation may have on the character of tuberculosis in the guinea pig. The simplification of the experimenter's work is also very considerable.

A preliminary experiment sufficed to show that this mode of administration of testosterone propionate in the guinea pig had the desired effectiveness. Implantation of one or more pellets in young guinea pigs, with a total absorption of 2–3 mg. of the drug in two weeks or less, resulted in increases in the weight of the prostates of 100 per cent and of the seminal vesicles of 300 per cent as compared to the control.

In the present experiment the evidence of effective dosage is not so striking in the comparison of prostatic and seminal vesicle weights at the end of the experiment. This can be accounted for by the growth of these organs in the control animals, finally approaching the size at which the dosage of hormone was maintaining them in the treated group. A reference to the figures for the preliminary group showed that the maximal growth of the prostate and seminal vesicle had taken place very early in the treatment.

The evidence of effectiveness of the drug is much more strikingly shown by the weight and histology of the testes. It is common knowledge that the gonads atrophy when the sex hormones are artificially supplied in large quantities. This is especially marked in young animals. It has been demonstrated with the use of several different androgens by Bottomley and Folley (31) in the young male guinea pig. On the basis of this evidence the effectiveness of the hormone is unequivocally demonstrated in every animal. The significance of this effect is also worthy of some consideration. Moore and Price (32) early showed that this atrophy of the gonads with

sex hormone administration could be prevented by simultaneous administration of a gonadotropic preparation. Bottomley and Folley confirmed this in their guinea pigs. It is quite apparent in figure 3 that the testis of the hormone treated animal is essentially the same histologically as that of the much younger immature animal. It has been shown by Smith (33) that the result of hypophysectomy in the male animal is a regression of the testis to the immature state. The assumption seems justified, therefore, that the failure of gonadotropic secretion by the hypophysis is one effect of androgen administration. If this is true, then our experimental animal has been one largely deprived of gonadotropic hormone during the period of infection. This is of some interest in view of the attempts to influence the course of experimental tuberculosis by administration of gonadotropic hormones (16, 19, 22, 23).

The experimental data do not indicate any effect of the hormone treatment on the extent or character of the lesions. The average extent of dissemination of the lesions is practically identical when the spleen, liver, lungs, and bronchial lymph nodes are taken together. A greater amount of tuberculosis in the spleens of the treated group is balanced by a greater amount in the lymph nodes of the controls, whereas the amount in the lungs and livers of the two groups is nearly identical. No difference was appreciable in the local lesions such as Takamura (34) has described between young and mature guinea pigs. All the local lesions in our animals tended to be rounded, hard nodules, such as he described in his young animals. Although a few in each of our groups showed small ulcerations, the pronounced tendency to "necrosis, softening, and ulceration" which he described in his mature animals was not observed in any of ours.

The influence of age on the skin reactivity to tuberculin in the guinea pig has been studied by Freund (35) and his results confirmed by Valtis (36). Freund infected two day old animals and their mothers simultaneously and found that the severity of the skin reactions of the young animals increased as they grew older, although the mothers showed maximal reactions from the earliest test. The sexes were not separated. Although the author makes no point of it, an examination of his data shows a more uniform correlation of the degree of reactivity with body weight than with age. Of the eight

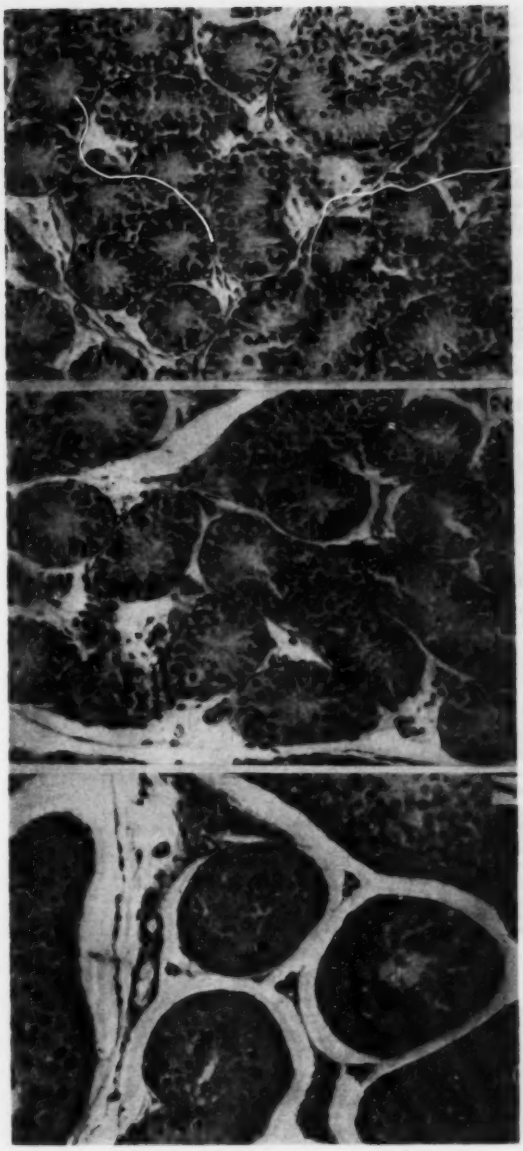

FIG. 3. EFFECT OF TESTOSTERONE PROPIONATE TREATMENT ON TESTES

(1) Animal no. 4, untreated control, age 49 days.

(2) Animal no. 7, total absorption of 7.0 mg. T.P. in 45 days, age 48 days.

(3) Normal untreated control, age about 20 days (no tuberculosis). (All magnifications ×230.)

animals that reacted perceptibly to one or another test, the average body weight was 206 grams (170–250 gms.) and of the fifteen non-reactors, the average weight was 140 grams (110–175 gms.). This same correlation of degree of reactivity with body weight is evident in the average figures in table 1, although there was considerable over-lapping between the individuals in our groups. These animals were also older and heavier at the time of this test than were Freund's and there were no completely non-reactive animals. The data give no indication that the skin reactivity was influenced by the hormone treatment.

The age differences in the character of human tuberculosis are dependent to an unknown extent on *acquired* resistance as contrasted to *native* resistance (10). The effect of estrogenic hormone on acquired as well as native resistance to tuberculosis in the adult female guinea pig was investigated by Gray and Brack (21) and no effect found. The brief period of immaturity in the guinea pig, during which the present experiment was conducted, did not permit the investigation of acquired resistance readily.

SUMMARY

Testosterone propionate has been administered to immature male guinea pigs, by subcutaneous implantation of pellets of the drug, during the course of their infection with tuberculosis. The animals were all sacrificed after the same period of infection together with the controls. No effect of the treatment was observed on the extent of distribution or character of the lesions nor on the degree of skin sensitivity to tuberculin.

We are greatly indebted to Dr. Arnold R. Rich for his advice in the execution of this experiment.

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SKIN, CONJUNCTIVAL AND SCLERAL REACTIONS IN THE COURSE OF THERAPY WITH SULFATHIAZOLE

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In the few years which have elapsed since the advent of the sulfonamide derivatives into the field of chemotherapy, there has been observed a multiplicity of untoward reactions. It was early recognized that the skin was frequently the site for a goodly group of these phenomena. Clinical reports have appeared concerning skin reactions to sulfanilamide, sulfapyridine and the other drugs of this series. As each new related compound has received a therapeutic test, it, in turn, has been watched to determine its capacity for producing one of the various types of dermatitis. Without exception every compound has exhibited this tendency so that now skin reactions are accepted as unwanted but inevitable.

The incidence of rash has not been low. Long and his co-workers (1) reported that they had observed skin disturbances in 1.6 per cent of 307 adults and 3 per cent of 101 children given sulfanilamide. Blake (2) reported that about 10 per cent of a series of patients ill with pneumonia and treated with sulfapyridine had rashes, a figure which was comparable to that previously published by him (3) for the incidence of dermatitis in patients ill with beta hemolytic streptococcus infections and treated with sulfanilamide. Volavsek (4) stated that about 3.5 per cent of his patients who were receiving sulfanilyl dimethyl sulfanilamide therapy developed some form of dermatitis. Consequently, we expected to see rashes with the institution of sulfathiazole therapy. We were not prepared to find them, however, as commonly as we actually did, for, in a group of 78 cases which received that drug for a period of more than a few hours, we observed 10 cases with skin eruptions (12.8 per cent).

The types of rash which have been observed with the older preparations have been numerous. They have been described as erythema-

tous, morbilliform, vesicular, bullous, urticarial, purpuric, exfoliative, to mention only a few of the terms which have been employed. From time to time it has seemed that one or another of the rashes might be distinctively associated with one of these products, as the morbilliform rash in the instance of sulfanilamide (5). Continued experience, however, has shown that such was not true. While one form of eruption might be somewhat more commonly observed during the course of therapy with a particular drug, another of the above-mentioned types of rash might be discovered at any time during the treatment of other patients with the same product. Fever, it has been noted (6), was a not uncommon accompaniment of the eruptions, and might antedate the appearance of the rash by hours or days. Moderate leucocytosis with a slight increase in the polymorphonuclear eosinophils also has been noted with these reactions.

As has been mentioned, we have observed 10 cases of skin reactions during the first three and one-half months that sulfathiazole has been in use in this clinic. We have felt that these reactions undoubtedly resulted from the administration of this drug. They all appeared after the compound had been prescribed for several days. No other obvious etiological agent could be discovered. All of the eruptions cleared rapidly, coincident with the disappearance of the drug from the blood stream. Furthermore, they were accompanied almost always by one or another of the previously mentioned companion signs of a sulfonamide reaction.

The rashes fall into three main groups: three were maculo-papular, five were "urticarial," and the remaining two were quite like "erythema nodosum." It seems worthwhile to describe these lesions a little more fully, for the last two groups may merge into each other, and certainly they are quite different from any of the eruptions which we have observed in this hospital with other derivatives of sulfanilamide. In patients showing the "urticarial" reactions the skin of the extremities was involved primarily, although the trunk was not spared when the rash became extensive. The involved area of the skin was raised, red, well outlined, and irregularly thickened. These areas were approximately 1 to 3 centimeters in diameter. Itching was observed but was generally neither conspicuous nor annoying. As the lesions faded, they left a reddened area at the site for a few days,

but even this soon faded. The reactions which have been likened to "erythema nodosum" were deservedly placed in that category. They may have been simply more advanced stages of the so-called "urticarial" lesions, but when they were viewed by individuals who were not prejudiced by knowledge of the subjects' medication, without exception they were described as typical of "erythema nodosum." They were thickened, slightly tender, red, raised lesions, some 2 to 5 centimeters in diameter, which occurred more commonly on the extensor surfaces of the legs and forearms, although some of them were observed on the thighs and upper arms. When these nodules regressed, however, unlike true "erythema nodosum," they failed to turn dusky reddish-blue, and they likewise failed to leave any local discoloration. One patient showed tenderness and swelling of the wrist and ankle joints. These promptly subsided with withdrawal of the drug.

One entirely new type of drug reaction has been observed in this group. So far as we have been able to determine, no similar lesion has been described with any of the other sulfonamide derivatives. This lesion is a conjunctival and scleral injection, restricted in the main to the exposed portions of the bulbar conjunctiva and sclera. The injection has been marked in six cases and there has been a considerable amount of burning and a watery discharge from the affected eye. Four of the six cases occurred in conjunction with a drug eruption, while the last two were seen as isolated phenomena. In at least three of the cases, the eye nearest the light was disturbed either solely or at least primarily. In two of the cases which were most exposed to light, the eruption and conjunctivitis were much more severe, involving the exposed surfaces and even the palpebral conjunctivae. In none was any "after-sensitivity" to light noted, *i.e.*, any recrudescence of the conjunctivitis on exposure to light after sulfathiazole had been discontinued. That this reaction did not invariably follow exposure to light is shown by the fact that several other patients were exposed to winter daylight during the course of sulfathiazole therapy without any untoward results. Figure 1 shows the typical triangular patch of conjunctivitis.

The diseases for which these patients received sulfathiazole constituted a heterogeneous group: pneumococcal pneumonia, lung

abscess, gonococcal arthritis, hemolytic streptococcal abscess, chronic staphylococcal parotitis and chronic staphylococcal osteomyelitis. The time of appearance of both skin and conjunctival reactions was between the fourth and sixteenth days of sulfathiazole therapy. Five (50 per cent) were noted between the tenth and twelfth days of treatment. Seven were accompanied by fever. There seemed to be no definite relationship between the eruption and the blood levels of free or acetylated sulfathiazole. None of the patients gave any clear history of previous allergic manifestations of any kind, although several were found to have exhibited reactions to related drugs, either previously or following the present reaction. No serious sequelae

FIG. 1

were noted. Nevertheless, the finding of an eruption was accepted as indication for the interruption of specific therapy. In two of the cases after the initial reaction had subsided, it was deemed advisable to continue chemotherapy with sulfapyridine. In each of these patients an exacerbation of the same type of skin reaction was noted. One had a recurrence of a maculo-papular eruption, while the other experienced an "urticarial" response again at the same sites as before.

In view of the apparent relationship of some of these reactions to exposure to light, the question of light sensitivity as an etiological agent in the appearance of sulfonamide reactions has been raised again. The possibility of such a relationship has been suggested before on

numerous occasions (7–10). Conclusive evidence of this relation to light is lacking, however. Tedder (11) suggested that these reactions be separated into three groups: 1) those precipitated by sunlight; 2) those due to sensitivity to the drug itself; and 3) those occasioned by poor tolerance or saturation with the drug, but showing no sensitivity to the drug. Epstein (12) agreed with Tedder's view without presenting good evidence to substantiate it. A case report has been published by Rogers (13) in which his patient was found to be sensitive to several local anesthetics. This individual later reacted very sharply to sulfanilamide, and it was discovered that these products had, as their common constituent, para-amino-benzene. Recently, Goodman (14) has reported some work which seems to afford experimental basis for this observation of Rogers. Goodman's patient was found to be sensitive to aniline and to allied compounds, but "there was consistently a negative response to chemicals of a benzene ring structure in which the amino group and the carboxyl group were in a position other than to constitute the structural base para-amino-benzoyl." He felt that the "offending factor might be the complete substituted aniline and not the simple benzene nucleus." Just what relationship may exist between skin reactions to these drugs, light sensitivity and chemical structure is not yet clear.

SUMMARY

Ten cases of skin eruptions and six cases of conjunctival reactions in a group of 78 cases treated with sulfathiazole are reported. So far as we have been able to determine, the conjunctival injection constitutes an entirely new type of reaction to the sulfonamide group of chemotherapeutic agents. The relationship of these reactions to exposure to light is discussed.

ADDENDUM

Recently we have observed a recurrence of the conjunctival injection in a patient to whom sulfathiazole was readministered. On the seventh day of his first course of drug therapy this patient began to run a low grade fever. On the eighth and ninth days of treatment there appeared a fairly marked conjunctival and scleral reaction such as has been described. This reaction was accompanied by a watering and burning sensation in the eyes. Within forty-eight hours after

the drug had been discontinued, the temperature had dropped to normal and the eyes had cleared completely. After a six day interval, one-half gram of sulfathiazole was given and twelve hours later one gram of this compound was again administered. A rather more severe reaction was observed in both eyes than had previously been noted, and it was accompanied by fever. This reaction became progressively more marked during the next twenty-four hours of therapy. The slight thermal reaction, as well as the conjunctival injection, subsided promptly when the drug administration was discontinued.

We wish to thank E. R. Squibb and Sons for providing the sulfathiazole which was used in treating this group of patients.

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TREATMENT OF LARYNGEAL PAPILLOMA IN CHILDREN WITH ESTROGENIC HORMONE

Preliminary Report

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Papillomata are common benign tumors of the larynx. They arise from squamous epithelium, do not infiltrate, and occur in adults, as well as children. The etiology is unknown, although Ullman (1) of Vienna transplanted the growths by a filterable agent. Although the adult and infantile papillomata seem similar histologically, there is a great difference in the behavior of these growths. In adults, papillomata are single, and complete removal results in a cure, although if not removed, papillomata may become malignant. In children, however, papillomata tend to be multiple and to multiply and implant themselves on other adjacent sites such as the trachea, the epiglottis or even the mucous membrane of the mouth and lips. Soon the larynx may be entirely filled with the growth and asphyxia may result unless a tracheotomy is performed. One can understand that, owing to this tendency to multiply and extend, the operative treatment of papillomata is not satisfactory.

Semon (2) mentions a case in which no fewer than 17 thyrotomies had been performed on the same patient with recurrence of the growth on each occasion.

Robinson (3) has recorded a case in which endolaryngeal operations were performed 19 times, tracheotomy 3 times, intubation 3 times, thyrotomy and laryngotomy. Death occurred suddenly, and in the post mortem examination it was found that papillomata filled the larynx.

Multiple papillomata in children have another strange characteristic. Suddenly the growths stop multiplying; the spreading and implanting characteristic stops, and the growths can be removed

with no recurrences. This usually takes place at about the time of puberty. With this experience in mind, Dr. Holt (4) undertook, by the injection of *Testosterone*, to bring on puberty in a small colored boy suffering from papillomata of the larynx, and although marked secondary sex changes resulted, the papillomata were not affected.

It is known that some definite change takes place in the larynx at puberty. It is common knowledge that a boy's voice changes. This is thought to be due to a sudden increase in the size of the larynx at this time, along with lengthening of the vocal cords. In order to detect any microscopical changes which occur in the epithelium and muscles during childhood and adolescence, Dr. Smith of the Department of Pathology, and Dr. Mulligan of the Department of Laryngology are examining microscopically larynges of children from two to sixteen years.

It has been shown by TeLinde and Brawner (5) following the work of Lewis that local applications of estrogenic hormones bring about a definite change in the vaginal mucosa of young girls.

Since papillomata tend to stop growing at around puberty, and since estrogenic hormone is known to have the power to change infantile epithelium to adult type epithelium, it was thought that local applications of the hormone to the larynges of children suffering from papillomata might be beneficial. Accordingly, four patients who had previously been treated surgically, and one new patient, were treated by weekly applications of an estrogenic hormone (*Amniotin-in-oil*, 10,000 International Units per cc., Squibb). The applications were made to the larynx through a direct laryngoscope, or by spraying the solution directly into the larynx. The following is a brief review of the five case histories:

C. K. 137091. White. Male. Unable to speak above a whisper since the age of two. Seen March 25, 1938, age seven years, and with a direct laryngoscope the entire larynx was found to be filled with papillomata. A tracheotomy was done and papillomata were removed on several occasions until February 29, 1939 when applications of *Amniotin* were begun. This was applied five times and on examination of the larynx on March 31, 1939, there was only a small amount of papillomatous growth left in the anterior commissure. The growth disappeared rapidly. On May 1, 1939, the patient was speaking in a clear voice and on re-examination October 13, only a small amount of granulation tissue was seen below the vocal cords. The larynx was otherwise perfectly clear. The patient was breathing well and the tracheotomy tube was removed on October 16, 1939.

Amniotin discontinued. When last seen, January 5, 1940, the larynx was still free of papillomata.

G. J. 102852. Colored. Female. Age 5 years. First seen March 17, 1937. A diagnosis of papillomata of the larynx was made. Tracheotomy was done March 19, 1937. Papillomata were removed on several occasions, but always recurred rapidly. The papillomata spread over the larynx and epiglottis and down into the trachea so that breathing became very difficult when the tracheotomy tube was removed. Papillomata were removed from the trachea on three occasions. On February 27, 1939, applications of *Amniotin* were begun and the throat was sprayed at weekly intervals for five weeks. On March 31, the papillomata were not spreading. They were beginning to diminish noticeably by April 14, 1939. Papillomata were again removed July 28, 1939. When seen December 29 the entire larynx was free except for a small piece of pedunculated papilloma in the anterior commissure. This was removed and the *Amniotin* discontinued. Tube removed, larynx clear, February 12, 1940.

F. B. 161408. White. Male. Age 5 years. First seen January 6, 1939 with a diagnosis of "web of the larynx." Examination of the larynx with the direct laryngoscope under general anesthesia failed to disclose any web, but showed both cords covered with papillomata. This tissue was removed and *Amniotin* was applied. A small amount of papilloma was again removed July 3, 1939 and *Amniotin* was again applied to the larynx. This was repeated in October and November. The larynx was now practically clear. The tissue removed on the last occasion showed a polyp but no papillomata. When last seen, December 29, 1939 (now six years old) the larynx was clear. *Amniotin* discontinued.

J. S. 146031. Colored. Female. Age 6 years. First seen July 1, 1938 when she was admitted to the hospital with a diagnosis of papilloma of the larynx, to have tracheotomy performed. Papillomata removed August 12, 1938, and on two other occasions. Beginning February 24, 1939, patient had *Amniotin* sprayed in the larynx. Examined with the direct laryngoscope on March 27, 1939: very little papillomatous growth remaining in the larynx, not enough to remove. She had in all, eleven applications of *Amniotin* and when examined November 19, 1939 the larynx was perfectly clear. *Amniotin* discontinued.

A. C. 104448. White. Female. Age 7 years. First seen April 5, 1937, diagnosis papilloma of the larynx. A tracheotomy was done on April 16, 1937. Papillomata were removed on many occasions, and the growths were fulgurated on February 11, 1938. A large mass was removed again January 11, 1939. On January 27, 1939, *Amniotin* treatments were begun, the larynx being sprayed with *Amniotin* on ten occasions. Papillomata were removed again May 22, 1939, and on October 6, 1939 the larynx was clear except for a small amount of tissue near the anterior commissure. The larynx was clear on December 29, 1939. *Amniotin* discontinued. Larynx clear, tube removed February 12, 1940.

Papilloma of the larynx is a self-limited disease. The factors which cause spontaneous regression are unknown. In the past many kinds of treatment have been used without interrupting the natural course of the disease: excision, x-ray and radium, fulguration, local application of chemical caustics, local and subcutaneous administration of testosterone. It seems significant that in all of the 5 cases here reported the papillomata cleared up after about the same period of treatment, in spite of the fact that the children varied in age from 6 years to 10 years.

It is estimated that 0.1 cc. (1000 International Units) of the estrogenic hormone was applied to the larynx at the time of each treatment. This dosage did not cause any constitutional disturbance and no secondary sex changes were noted.

SUMMARY

In this preliminary investigation local application of estrogenic hormone (*Amniotin*) seems to have had a definitely beneficial effect in the treatment of multiple laryngeal papillomata in 5 children. The characteristics of the growth change from those of the spreading infantile type to the adult type. This form of treatment is simple and the results have apparently been more satisfactory than those obtained in the past.

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PROCEEDINGS OF A MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

Held in the Hurd Memorial Hall, March 11, 1940

The President of the Society, Dr. G. Canby Robinson, presided. Abstracts of the papers and discussions follow:

Studies of Cardiac Enlargement in Children with Rheumatic Heart Disease. By Helen B. Taussig and Marcel Goldenberg. (Department of Pediatrics, The Johns Hopkins Medical School.)

This study is concerned with changes in the size of the heart which may occur in children with rheumatic heart disease. Our method is similar to Palmer's, *i.e.*, the superimposition of one X-ray upon another. The cardio-thoracic ratio was used to determine rate of growth.

The study shows that there are three distinct and separate sequences of events which may occur in rheumatic fever. First, the heart may show normal growth. Second, the heart after a period of enlargement, may remain stationary in size while the chest grows. Third, the heart may undergo progressive enlargement.

In *Group I*, it is natural to find that after one or more attacks of acute rheumatic fever with evidence of cardiac strain, but with no residual cardiac damage, the heart which has never been enlarged may show normal growth. We have seen 30 such cases. The same phenomenon has been observed in cases with valvular damage. In one case the patient had aortic insufficiency for 7 years. The heart was never enlarged; the increase in the size of the heart was commensurate with the growth of the chest. We have observed 9 such cases with mitral insufficiency, 3 with aortic insufficiency and 1 with mitral insufficiency and a mid-diastolic murmur.

In *Group II*, the heart was enlarged when the patient first came under observation; thereafter the heart remained stationary in size while the chest grew. We have observed 24 such cases in which the heart ceased to enlarge. Five had no residual valvular damage; 6 had mitral insufficiency; 6 mitral insufficiency and stenosis; 3 aortic insufficiency and 3 aortic insufficiency and probably mitral insufficiency and stenosis. In approximately one-half of the cases, the heart has come to lie within normal limits. After the size of the chest became commensurate with that of the heart, there was a resumption of normal growth.

In *Group III*, the cases have shown progressive cardiac enlargement. In those in which the process has extended over a period of years, the heart has enlarged out of proportion to the growth of the chest. We have observed 16 such cases. Two have mitral insufficiency; 3 have mitral and aortic insufficiency; 7 mitral insufficiency and stenosis; 1 mitral and aortic insufficiency and stenosis.

In the first two groups the mortality rate is approximately 10 per cent and 80 per cent are leading normal active lives. In the third group the mortality rate is 50 per cent and an additional 25 per cent are chronic invalids.

Conclusions. (1) Cardiac enlargement is not necessarily progressive in rheumatic fever even in the presence of persistent valvular damage.

(2) Enlargement occurs during periods of active rheumatic infection and comes to an end when infection subsides.

(3) In a number of cases with an active infection, cardiac enlargement came to an end when the patient was put to bed. This indicates that the load placed on the diseased heart is an important factor in cardiac enlargement.

(4) The difference in the mortality rate between the first two groups and the third group illustrates that progressive cardiac enlargement is a very serious phenomenon.

DISCUSSION

Dr. E. A. Park said that he had been following Dr. Taussig's studies with a great deal of interest. He thought that it was an entirely new idea that the heart of a rheumatic child should enlarge for a period and then remain stationery in size, until the body caught up with it. These studies seem to give an objective basis for prolonged bed rest, in the treatment of rheumatic heart disease. It has been impossible to determine by statistical methods, just how valuable bed rest really is. Bed rest has been employed largely because of the fact that it has been found beneficial in the treatment of analogous diseases, such as tuberculosis. It is interesting that in Dr. Taussig's work, in the main, the children whose hearts ceased to hypertrophy were the ones who were obedient in regard to treatment, and the ones whose hearts went on to become progressively larger were the ones who did not cooperate.

Dr. W. T. Longcope remarked that Dr. Taussig's paper seemed to uphold an idea which he and Dr. Swift frequently discussed many years ago, namely that patients with rheumatic heart disease should, logically, be treated by prolonged sanatorium care, such as is received by patients with tuberculosis. He deplored the fact that there were so few sanatoria for the treatment of rheumatic heart disease—none in Maryland.

Dr. Taussig, in closing, said that she had neglected to emphasize, in giving her paper, that in the cases in which the heart had been only slightly enlarged, it remained stationery in size until the increase in the size of the chest had reestablished the normal cardiothoracic ratio. From this point on the heart and thorax grow along together.

The Experimental Production of Cirrhosis of the Liver by Means of a Deficient Diet. By ARNOLD R. RICH and JOHN D. HAMILTON. (Department of Pathology, Johns Hopkins Medical School.)

See *Bulletin of The Johns Hopkins Hospital*, page 185, March, 1940.

DISCUSSION

Dr. E. A. Park asked what other experimental means had been successfully employed for producing cirrhosis of the liver.

Dr. Rich replied that scarring of the liver following necrosis had been produced by infection, and by toxic agents, such as chloroform and arsenic. Cirrhosis has been reported to occur in animals kept alive for long periods by the administration of insulin following total pancreatectomy.

Dr. Longcope commented upon the effect of alcohol, in making the liver more susceptible to damage by toxic agents. He wondered whether this action of alcohol, combined with vitamin deficiency, might not contribute to the production of so-called alcoholic cirrhosis.

Dr. Hugh W. Josephs asked whether alcohol might not have something to do with the destruction of ingested vitamins.

Dr. Rich said that he thought that this was possible, but would apply only to vitamins shown to be destroyed by alcohol.

Dr. W. Halsey Barker asked whether liver extract had been substituted for yeast in any of the experiments, and whether blood counts had been made.

Dr. Rich replied that experiments were now being carried out, in which liver extract is being substituted for yeast. Blood counts were not done routinely in the experiments reported but in one animal it was found that there was an anemia with marked macrocytosis.

Dr. Henry M. Thomas, Jr. commented upon the low serum protein and asked whether Dr. Whipple's animals with a low serum protein showed any changes in the liver.

Dr. Rich replied that he did not know, but that, so far as he was aware, no changes in the liver had been reported, nor were cirrhotic changes reported in the studies of Weech on dogs with low serum proteins.

Rat Bite Fever with Arthritis due to Streptobacillus Moniliformis. By THOMAS MCPHERSON BROWN and JOHN C. NUNEMAKER. (The Biological Division of the Department of Medicine, Johns Hopkins Medical School.)

A 49 year old white farmer was bitten by a rat and 16 days later was admitted to The Johns Hopkins Hospital because of fever and arthritis. The streptobacillus moniliformis was isolated from the joint fluid and the blood. Severe arthritis, localized in the left knee (destructive changes by X-ray), and laryngeal involvement (drawing to illustrate) were the chief clinical features. No definite improvement from aspirin or sulfanilamide; apparent benefit with gold therapy. Streptobacillus moniliformis has been agglutinated in high titre by patient's serum during four months period, also by serum from immunized rabbits.

L_1 pleuropneumonia-like organism of Klieneberger has been isolated from cultures of streptobacillus moniliformis.

Migratory polyarthritis has been observed in 100 per cent of mice injected both intravenously and intraperitoneally with organism isolated from the patient.

The streptobacillus moniliformis and the L_1 organism have been recovered from the blood of experimentally infected mice, also from affected joint of a mouse having persistently negative blood cultures. The experimental arthritis is illustrated by photographs, X-rays, and sections of the joints; also by diagrammatic drawings of the joints involved, indicating the degree of involvement and the migratory character of the arthritis.

DISCUSSION

Dr. Longcope said that the streptobacillus moniliformis and other pleuropneumonia-like organisms appeared to be growing in importance. These organisms lie midway between bacteria and filterable viruses. Many of the diseases caused by filterable viruses and the pleuropneumonia-like organisms differ from the usual bacterial diseases in that they start as diseases of animals and are transmitted from animals to man. He asked whether other investigators had been able to produce arthritis in animals with the streptobacillus moniliformis obtained from humans.

Dr. Brown replied that arthritis had been produced in animals by Levaditi with a human strain of streptobacillus moniliformis. However, this is the first time arthritis has been produced in mice with a human strain, although it has been produced in mice with animal strains.

Dr. Chesney asked why it was that the streptobacillus moniliformis could not be transferred from cultures with an ordinary wire loop.

Dr. Brown replied that this applied only to the early growth of the organism. After a number of subcultures the organisms can be transferred by ordinary methods, but in the early cultures it is of the utmost importance to make the transfers early and by using cutouts. With the L_1 organism the cutout method is important because the colonies are extremely small, the organisms are very friable and the growth is usually imbedded in the culture medium.

Dr. Wainwright said that the arthritis with which Dr. Brown had been dealing was evidently a metastatic arthritis which, in some respects, resembled rheumatoid arthritis, but that there were other respects in which it was unlike rheumatoid arthritis. He said that the agglutination of Group A streptococcus by the serum of patients with rheumatoid arthritis was such a characteristic feature of the disease that he wondered whether Dr. Brown had studied this property of the serum in his patient.

Dr. Brown said that such a study had been made; that the serum of his patient agglutinated Group A streptococcus, in quite high titer, but did not agglutinate streptococci of Groups B and C.

Dr. Wainwright said that he thought that this was a very significant observation, as indicating an analogy between rheumatoid arthritis and the malady from which the patient suffered; that it should be remembered that the agglutination of Group A streptococcus in rheumatoid arthritis may not be a true antibody reaction.

Dr. Rich asked whether animals could be infected with the streptobacillus moniliformis by feeding the organism, as well as by injecting it. He thought that this was of some importance in connection with the milk-borne epidemic of Haverhill fever.

Dr. Brown said that such experiments were planned but had not yet been carried out.

Cancer of the Breast. By I. RIDGEWAY TRIMBLE. (Department of Surgery.)

The importance of this question becomes strikingly apparent when we realize that approximately 40 per cent of the total mortality from cancer in the female is due to cancer of the breast. Should patients suffering from this condition be subjected to mutilating operations? Should they receive radium or x-ray therapy before or after operation? Should radiation therapy include sterilization? Personal communications received from the heads of some of the more important clinics in this country give diverse answers to these questions.

Radical resection of the breast, employing the most painstaking technique, is the most effective weapon against cancer of the breast. Scrupulous attention to fine operative detail, according to principles originally laid down by Halsted, will make the difference between success and failure.

While cures may be effected in approximately 75–80 per cent of cases by this type of operation when there are no metastases, the figure drops to about 25 per cent in patients who already have metastases. In 80 per cent of the patients presenting themselves for examination with cancer of the breast metastases have already occurred. Radiation following operation has failed thus far to increase perceptibly the percentage of cures.

Because of the still unsatisfactory percentage of cures achieved by a radical operation plus postoperative radiation in patients suffering from cancer of the breast with metastases, and because preoperative radiation is known to bring about marked reduction in size of the original growth and of the glandular metastases, adequate preoperative radiation should be employed in many cases.

DISCUSSION

Dr. Curtis F. Burnam remarked that a thorough consideration of breast-cancer therapy, aiming at improvement in results, is much to be desired. As with most serious diseases, the clinical picture is a variegated and very complex one. Enormous differences in rate of spread, response to radiation and histological appearances exist. Ordinary clinical signs are usually adequate in diagnosing cancer but not the type. Histological examination is frequently necessary to differentiate from benign conditions.

Everyone agrees that the earlier therapy is instituted, the better the result. Every effort should be made to take fear out of people and teach them that in the beginning the disease is curable. There is abundant evidence that injudicious or poorly applied therapy, whether surgery or radiation, especially in the advanced cases, may be actually harmful in the sense of shortening life and increasing discomfort.

Existing statistical studies of the results of surgery are fairly reliable. They vary considerably from clinic to clinic, which is to be expected when the limits of operability vary so much from surgeon to surgeon, and when the mathematical laws of probability are taken into account.

It is surprising that so many reports state that post-operative radiation has not materially improved results. One cannot help but question the validity of such observations because it is common experience to see clinical cures effected after recurrence has taken place and also in cases where all the disease is not removed at the time of operation. A number of such cases have come under my observation, and the healing in some instances has persisted for years.

No group of tumors varies more in sensitivity to radiation than breast cancer; the acute inflammatory types are almost as sensitive as lymphosarcoma. On the other extreme, there are cancers in which such high dosage has to be employed that radiation is practically useless because of the impossibility of protecting normal tissues. There is no question that in certain breast cancers where adequate radiation has been given, a complete clinical cure is obtainable; however, these do not constitute a majority of the cases. In my own experience among these cancers cured by radiation alone there are those in which the disappearance has persisted for some years.

It is questionable whether radiation of the ovaries in women during the menstrual life is of value in the treatment of cancer of the breast. It seems to me that further clinical or laboratory evidence should be offered on this point before anything like a general adoption of the method.

If operative and radiation methods are to be integrated, a plan should be developed for each patient before any treatment is resorted to. It should constantly be borne in mind that treatment cannot be by rule of thumb. So far as radiation is concerned, it is perfect nonsense to set down a plan of so many R-Units to so many fields, and to adhere to this with all patients. Under such a plan some patients would be satisfactorily treated—some over-treated and others under-treated. Actual observation and consideration of changes during treatment offer the best guide as to the amount of radiation that should be given.

I hope Dr. Trimble will go on with this subject and set up a comprehensive plan of treatment and study of breast cancer, utilizing both operation and radiation. A promising combination, particularly in the advanced cases, is pre-operative radiation, followed by operative surgery. For this combination there is abundant clinical and experimental support of a convincing character.

Dr. J. W. Pierson said that two years ago his department had reviewed all of the available literature on the treatment of carcinoma of the breast. As Dr. Trimble had said, it was found that in about 20 per cent of the cases the tumors were localized to the breast; in such cases the patients had a 4 out of 5 chance of being alive after a period of 5 years. When the carcinoma has invaded the axilla, the chance is reduced to 1 out of 5; when it has gone beyond this, the chance is further reduced to 1 out of 20. These figures are based on operative treatment alone.

When surgery was supplemented with radiation, salvage increased 10 to 15 per cent in this second group. There was no increase in salvage in the third group.

In each individual case, the surgeon and radiologist, together, should work out a plan of treatment. The technique and dosage of irradiation are just as important as the technique of surgery. Dr. Adair's experience with 117 carefully studied cases has clearly emphasized the importance of adequate dosage. It is important for the surgeon to realize that it takes time to give an adequate dose and due allowance must be made for this in the plan of treatment.

Dr. Grant E. Ward said that during the past several years he had been following a carefully pre-arranged schedule of treatment, such as that suggested by Drs. Burnam and Pierson. This schedule included preoperative irradiation. The latter makes necessary certain minor alterations in operative technique, for in the irradiated area there are adhesions and the tissues are more vascular. The operation is necessarily bloodier and in these cases good use may be made of electro-surgery, for the control of hemorrhage. The added difficulties are well compensated for by the benefit which patients receive from preoperative irradiation. Massive tumors may melt away to such an extent that surgery is actually made easier. Large axillary glands may also be reduced in size, facilitating operative removal.

Some radiologists have claimed that irradiation closes the lymphatic channels of the breast and axilla, reducing the likelihood of metastases. However, there is, as yet, no proof of this. Dr. Ward said that he had observed open lymphatics in the axilla upon microscopic examination of tissue removed at operation after previous irradiation. It would be well worth while to carry out studies on experimental animals to determine the effect of irradiation upon the lymph channels of the breast and axilla. He suggested injection of a non-sclerosing radiopaque substance into the lymphatics of both breasts of female dogs and taking radiographs. Using one side as a control without irradiation, the other could then be treated by roentgenotherapy in a manner comparable to the preoperative treatment given for cancer of the breast. In 6–8 weeks after completion of such treatment, he suggested reinjection of the lymphatics and roentgenographs to compare the condition of the lymphatic channels. Also, the lymphatics of the breast and axilla should be studied microscopically.

Dr. G. G. Finney said that from the surgeon's viewpoint it was frequently difficult to make the pre-operative plans which Dr. Burnam and Dr. Pierson had suggested because there seemed to be as many plans of radiation as there were radiologists. He stressed the work of Dr. Hugh Trout of Roanoke who has combined a truly radical breast operation with a consistent plan of pre-operative, operative and post-operative radiation. Dr. Finney felt that it was important to work out a standardized technique and adhere to it as rigidly as possible, in order that results might be properly evaluated.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Medical Entomology. By WILLIAM B. HERMS. 3rd edition, 582 pp. Illus. $5.50. *The Macmillan Company, New York, 1939.*

The third edition of Herm's *Medical Entomology* is a thorough revision of the first and second editions of *Medical and Veterinary Entomology*. It is a 582 page book, divided into 24 chapters. The first six chapters are more or less introductory in nature. In them the author gives a brief historical account of the development of medical entomology; he discusses the relation of other fields of science to medical entomology, and emphasizes the value of experimentation with disease transmitting insects, as well as the need for ecological studies before the application of control measures; he includes definitions and discussions of parasites, hosts, types of parasitism, and host-parasite relations; he outlines the rôle of Arthropods in human and animal diseases; he describes insect and arachnid morphology, development, classification, and the structure of various types of insect mouthparts. The orders of insects are classified according to their mouthparts.

Chapters VII–XIII deal with the families of insects and arachnids that are of medical and veterinary importance. The principal morphological characteristics of each group are given, followed by a summary of the life cycle and habits, distribution, relationship to diseases, and control measures. The development of the pathogenic organisms and the diseases caused by them are also discussed. The following groups of insects are treated in these chapters: cockroaches, beetles, bedbugs, Reduviid bugs, sucking and biting lice, blackflies, sandflies (*Phlebotomus* and Ceratopogonids) and non-biting gnats, mosquitoes, horseflies, snipe flies, the house fly and its allies, tsetse flies, the stable fly, horn fly, louse flies, fleas, ticks, mites, spiders, and other venomous Arthropods. In these chapters keys are given to a few families of beetles, to the species of North American Cimicidae, to some of the species of Reduviidae, to families of North American Hemiptera and Homoptera that contain predacious species, to families of Diptera that are of medical importance, to the genera of North American mosquitoes and to adults, larvae, and male terminalia of species of *Anopheles* in the United States, to the genera of Nearctic Tabanidae, and to the families of Siphonaptera.

Chapter XVIII is a 30-page review of the various types of myiasis. Chapter XXIII, "Venomous and Urticarial Arthropods," discusses insect venoms, and includes a detailed review of the appearance, distribution, habits, and pathogenicity of the black widow spider. The final chapter deals with the use of certain Arthropods in medical practice.

The book has many excellent illustrations, but the reviewer believes that the

inclusion of additional aids—keys or tables—to the identification of species of disease-producing insects, would have increased the value of the book. The field of medical entomology has progressed so rapidly that it is impossible to give many details in a book of this size. However, at the end of each chapter there is a rather complete bibliography, which should be of value to those interested in a more intensive study of the field. Fortunately the author added the new advances in medical entomology without sacrificing the veterinary material that was present in the first and second editions; this part of the field is largely ignored in books dealing with disease-producing insects. Because of this, the third edition contains 189 pages more than does the first edition, and 120 more than the second edition.

L. E. R.

Diseases of the Skin. By RICHARD L. SUTTON AND RICHARD L. SUTTON, JR. 10th ed. revised. 1549 pp. Illus. $15.00. *C. V. Mosby Company, St. Louis, 1939.*

In the tenth edition of this well known book the authors have added 10 color plates, 340 new illustrations, 100 per cent increment in the number of words and 7000 bibliographic entries. The very large bibliography which reaches well into 1939 will be particularly helpful to the research student. The photographs are on the whole excellent and well chosen and the addition of new syndromes and rare diseases makes the book unusually complete. Some original ideas concerning the cause and treatment of a few dermatoses have been expressed; those referring to acne being of particular interest. If the treatment of this disease should prove successful in the hands of others the contribution will be indeed of great benefit. The book on the whole has a great deal to offer to the general physician and specialist as well as to the student and research worker.

L. W. K.

Lehrbuch der Augenheilkunde. By ERNST FUCHS. 16th ed. Revised by Dr. Adalbert Fuchs. 904 pp. Illus. Paper, 27 marks; bound, 30 marks. *Franz Deuticke, Wien, 1939.*

Among text books of ophthalmology, that by Hofrat Ernst Fuchs occupies a unique position analogous to Osler's Principles and Practice of Medicine. The first edition appeared in 1889, four years after Fuchs at the extraordinarily young age of 34, had been called to the chair of ophthalmology at Vienna. Later he used to say with a benign twinkle that he had written his text book because he had been laid up with sciatica, and that both the sciatica and the recurring editions of his text book returned periodically for visits, "like old friends." Fuchs held the chair in Vienna for thirty years but continued his clinical and pathological studies and writings for fifteen years after his retirement until his death in 1930. During this period the text book went through twelve editions at his hands and numerous translations into almost every modern language.

Fuchs' contributions to ophthalmology were in the domain of morphological pathology and of clinical classification. In respect to diseases of the anterior

ocular tissues he carried these studies forward in masterly fashion, achieving in many instances a synthesis of the two lines of study so that his clinical classifications were well buttressed by pathological evidence. The results of these analyses were embodied in the successive editions of his text, written in a style of rare lucidity and simplicity. It is no wonder that this book became the bible of students of ophthalmology for a generation. For many years the only serious competitor was the book by de Schweinitz in which diseases of the posterior ocular tissues were more extensively treated, and the relations of ophthalmology to internal medicine were more strongly emphasized.

The publication of an adequate posthumous edition of a text so intimately identified with a brilliant personality is a matter of considerable difficulty. In this case the difficulty is greatly increased by the fact that the science of ophthalmology has progressed beyond the solution of morphological and nosological problems to problems of pathogenesis and pathological physiology. Hence, the editor must decide, if he adheres to the point of view of the original text, to leave out a considerable part of modern ophthalmology, for if the newer viewpoint is to be presented adequately, the whole text would have to be re-written. Since the book passed out of the hands of the original author, three editions have been piloted by Salzmann. Salzmann's attitude appears to have been one of extreme caution. He enriched the volume with improved illustrations of fundus conditions, but on the whole, the text deteriorated appreciably in his hands.

Now the editorship has been transferred to Adalbert Fuchs, son of the original author, and like his father, professor at the University of Vienna. In attempting to re-establish the original character of the book, the new editor has made only the most modest additions. The section on fundus disease has been given a much needed expansion, but in the expansion the morphological and classificatory attitude of the original has been maintained. The section on extra-ocular muscles, a subject in which continental ophthalmology has been woefully backward, is re-written. Apparently, in deference to local fashion, the description of hereditary eye diseases is said to be expanded though the changes here are insignificant. The illustrations are much improved by inclusion of many plates from Adalbert Fuchs' Atlas of Histopathology of the Eye. In this way the great classic of a generation ago is given a slightly palsied continued existence, while a modern text book of ophthalmology remains to be written.

J. S. F.

Textbook of the Principles and Practice of Nursing. By BERTHA HARMER AND VIRGINIA HENDERSON. Fourth Edition, Revised. 1047 pages. Illus. $3.00. *The Macmillan Company, New York, 1939.*

This volume by Miss Henderson represents a complete revision of the textbook by Miss Harmer which has been a favorite since the first edition was printed seventeen years ago. The Curriculum Guide, published by the National League of Nursing Education in 1937, has greatly influenced the arrangement of subject matter.

Throughout the entire text prevention of disease is given as much emphasis

as its treatment and nursing care, and more stress has been laid on health and sickness in the community. The approach is always from health to sickness. For example, the care of the skin in health and the normal diet are taken up first and then the adaptation in sickness is presented. The subject matter has been divided into five parts, as follows: Nursing and Community Health Service; Fundamentals of Nursing Care; Assisting with Diagnostic Procedures; Assisting the Physician with Therapeutic Measures; and Introduction to Medical and Surgical Nursing.

An Appendix includes the following subjects: cleaning and stain removal, special enemas, special types of diet, weights and measures, and abbreviations and symbols.

Suggested readings are listed at the end of each chapter along with writings to which direct reference has been made. A great many excellent illustrations have been added which help to clarify nursing techniques and procedures.

There is a great deal of new material in the text. One of the most interesting chapters pertains to the control of communicable diseases. Most of the tests with which nurses assist, such as x-ray, basal metabolism, gall bladder and the dilution-and-concentration test of kidney function, have been fully described and explained.

In arranging the subject matter, Miss Henderson had in mind the usefulness of the text not only to the student nurse and the instructor of nursing but to the practicing nurse in fields outside of the hospital. It is undoubtedly one of the best texts available to nurses today.

V. B.

Ophthalmology. By BURTON CHANCE. 240 pp. Illus. $2.00. *Paul B. Hoeber, Inc., New York, 1939*. (Clio Medica XX.)

This little book is a welcome addition to this series of "Primers" of medical history. It covers ophthalmology from the earliest known records in the Sumerian, Babylonian and Assyrian ages right up to the present time. Although the entire subject of the history of ophthalmology is covered it is unfortunate that the limitations of space have made it necessary to be so very brief, particularly in the early chapters, parts of which are little more than an expanded outline. There are relatively few illustrations, only six in all.

The book is written in a very popular vein with adequate explanations of even the simplest ophthalmological conditions so that it should be readily intelligible to any layman. Frequent references to the author's own possessions among rare and historical volumes and to his acquaintance with leaders in ophthalmology add an informal personal touch. The author explains in his preface the complete lack of bibliography as due to lack of space.

This little volume is very interesting and light reading which should be entertaining to all those interested in medical history both inside and outside the immediate field of ophthalmology. However, it is not, nor does it pretend to be, by any means an exhaustive treatise.

J. M. M.

The Medical Career. By HARVEY CUSHING. 302 pp., $2.50. *Little, Brown & Co., Boston, 1940.*

These essays consisting of addresses delivered before various colleges and medical societies, are followed by a number of biographical sketches including those on Dr. Welch, Dr. Halsted and Dr. Councilman.

The addresses which are offered before audiences made up not only of medical men, discuss the general program in the education of students of medicine and emphasize the importance not only of their preliminary teaching but especially of the inclusion in their preclinical courses of some idea of the disturbances of bodily functions and even of bodily structure in those who are ill and require the care of a doctor. This should give a more vivid interest in physiology and anatomy and pathology than the isolated study of those subjects. In all, he feels that such training should fit the student at graduation for a career as a family doctor—in other words, he stresses the importance of the humanitarian attitude in medicine and would prefer that narrow and intensive specialization should follow only after that in the case of those especially fitted for it.

The biographical sketches are charming and give pleasing pictures of the early days, the careers and personalities of Dr. Welch, Dr. Halsted and Dr. Councilman. That of Haller is full of impressive records of that remarkable man.

The address at the opening of the Welch Library is particularly interesting in expressing his intense interest in the stirring influence of such a library and its impulse toward his favorite study of the history of medicine to which he has contributed so much.

W. G. M.

Primer of Allergy. By WARREN T. VAUGHAN. 140 pp. Illus. $1.50. *C. V. Mosby Company, St. Louis, 1939.*

This book, as Dr. Vaughan states in his preface, is "intended primarily as a guide for the patient seeking quickest relief from his illness." The two opening chapters are devoted to the explanation of the allergic nomenclature in such a manner as to be easily understood by the laity. The third chapter is an entertaining allegory explaining the "theory of allergy." The next three chapters are allotted to the methods of identifying the "enemies" (allergens) and to the methods of attacking (hyposensitization) the "enemy." In the seventh and eighth chapters Dr. Vaughan is speaking to his patients. Any allergic individual and any physician interested in allergy will find each sentence in these chapters full of useful information. They represent a fitting climax for his valuable book. The ninth chapter is a critical survey of what the patient may expect from proper treatment. The tenth and final chapter is devoted to detailed instructions which are of inestimable worth to a patient afflicted with any of the diseases of allergy. The chapters are cleverly titled and frequently headed by amusing cartoons.

E. L. K.

CARDIAC ARRHYTHMIA DURING CHEYNE-STOKES RESPIRATION

EDWARD MATTHEWS[1] AND W. BARRY WOOD, JR.

From the Cardiographic Laboratory of The Johns Hopkins University, and the Medical Clinic of The Johns Hopkins Hospital

Submitted for publication March 15, 1940

It has been known for many years that variations in the rate of the pulse may accompany the phases of Cheyne-Stokes respiration. It is not generally recognized, however, that this arrhythmia may be of such degree that the pulse rate at one moment in the cycle may be but half that present at another moment, and that changes in the cardiac mechanism, as well as in the rate, may be demonstrated electrocardiographically.

The phase of the Cheyne-Stokes cycle during which the arrhythmia becomes manifest is not the same in all cases. It was shown by Eyster (14) and later by Pollack (32) that, in Cheyne-Stokes breathing due to increased intracranial pressure, the slower pulse occurred during apnea, whereas in the Cheyne-Stokes breathing of cardiac or arterial disease the opposite was true, that is, the slower pulse was present during hyperpnea. The experiments of Cushing (10) afford explanation for the variations in pulse rate associated with the periodic breathing of increased intracranial pressure. These studies showed the periodic breathing to be secondary to fluctuations in blood pressure, a fall in blood pressure causing apnea, and a rise resulting in return of respiration. Since the pulse rate decreased as the blood pressure fell, apnea was found to be associated with the slower rate.

The arrhythmia of the heart during Cheyne-Stokes breathing of cardiac or arterial disease, however, cannot be so readily explained. It seems worthwhile, therefore, to present observations made in 4 cases of this nature, and to review the reports that have appeared in the literature.

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CASE REPORTS

Case 1. T. D.

A colored male janitor, age 69, was admitted to The Johns Hopkins Hospital (History No. 154078), October 4, 1938, complaining of shortness of breath. His health had always been good in the past. He had pneumonia at age 19, a penile sore at 20, without other manifestations suggesting syphilis, and urethritis at 30. The present illness began 10 months before admission, with exertional dyspnea soon followed by orthopnea, paroxysmal nocturnal dyspnea, swelling of the ankles, and nocturia. A physician had prescribed a green liquid medicine, 20 drops 3 times daily, which the patient continued to take until the time of hospitalization.

Examination revealed orthopnea, cyanosis, engorgement of the cervical veins, and pitting edema of the lower extremities. The respirations were Cheyne-Stokes in type with apnea of about 25 seconds and hyperpnea of 60 seconds. The retinal arteries appeared narrowed and there were small patches of white exudate near each macula. There were moist rales at both lung bases.

The pulse presented a cyclic irregularity. During apnea, the rate was about 100 per minute. At the beginning of hyperpnea, there was a rather abrupt change to a rate of about 50 which persisted till well into hyperpnea, when it quickly returned to the former rate. The peripheral arteries were thickened. The arterial blood pressure was 160/110; the venous pressure was 200 mm. saline by the direct method. The cardiac impulse was located in the sixth left interspace, 13.5 cm. from the mid-line. A double thrust was easily discernible. By percussion the area of cardiac dulness was found greatly increased, measuring 4.5 cm. in the fourth right space, 14 cm. in the sixth left, and 7 cm. at the base. There was a short systolic murmur at the apex and a protodiastolic gallop over most of the precordium. The second sound in the aortic area was loud and tympanitic. With the change to a slow cardiac rate at the onset of hyperpnea, the gallop disappeared and the second sound became widely split. With transition to a faster rate, the gallop returned and splitting of the second sound ceased.

The liver was enlarged downward 4 cm. below the costal margin. There was no evidence of ascites. A small scar was noted on the penis. Hemorrhoidal tags were present, and the prostate was found to be indurated and slightly enlarged. The neurological examination was negative.

Laboratory Examination. The serological test for syphilis (Eagle) was negative. The blood showed no anemia, and the total and differential leukocyte counts were normal. Examination of the urine was negative except for 1+ albumin and an occasional hyaline cast. The non-protein nitrogen of the blood varied from 50 to 37 mgm. per cent. The CO_2 combining power was 46.6 vol. per cent. Phenolsulphonephthalein excretion was 70 per cent in 2 hours. Roentgenograms revealed the heart markedly enlarged to the left and the aorta dilated and tortuous.

Routine electrocardiograms were taken at frequent intervals. Left axis deviation was constant. There was prolonged auriculoventricular (A-V) conduction.

On admission, October 4, the P-R interval was 0.32–0.36 seconds; after one week without digitalis it had decreased only to 0.30. Later, on October 28, after digitalis had been given in fairly large dosage, it increased to 0.40, and with a maintenance dose of 0.1 gm. powdered digitalis daily, it varied between 0.30 and 0.36. At various times complete A-V block occurred during the hyperpneic phase of the Cheyne-Stokes cycle. Intraventricular conduction was delayed, the QRS measuring 0.12 seconds. The QRS complexes were thickened; $S\text{-}T_{2\,\&\,3}$ were elevated, and the T-waves were in opposite direction to the major QRS deflection, so that the configuration of the ventricular complexes approached that of the common type of bundle branch block. In the chest lead (CF_3), the R-wave was absent. Studies of the cardiac arrhythmia are discussed below.

Therapy and Course. The usual regime for congestive heart failure was followed. However, because of the history of probable digitalis medication and the electrocardiographic evidence of A-V conduction defect, the administration of digitalis was withheld. At the end of one week the clinical condition was unimproved. As the P-R interval was still 0.30 seconds, it was felt that the block was probably organic rather than due to the drug, so the administration of powdered digitalis was begun and a total of 3.4 gm. given in the next 2 weeks. There was still no improvement and, in spite of frequent injections of caffein sodium benzoate and of aminophyllin, the Cheyne-Stokes respiration continued. The P-R interval increased to 0.40, and, on October 26, the cyclic complete heart block, which had not been present since the first day, returned and was present intermittently for the next few days. Venesection was done and, after the administration of ammonium chloride, 3 injections of salyrgan were given. A diuresis resulted, and steady improvement commenced. Finally, by the sixth week of hospitalization, all of the signs of congestive heart failure had disappeared, the blood pressure had fallen gradually from 160/110 to 118/72, and the respirations were no longer of Cheyne-Stokes type. There had been a loss in weight of 40 lbs. At the end of the eighth week the patient was discharged from the hospital with cardiac compensation restored.

Three weeks later, having failed to limit his activities, the patient was readmitted, again in congestive heart failure. The respirations were Cheyne-Stokes in type, but there was no evidence of cyclic change in the cardiac rhythm. With the use of mercurial diuretics, improvement was rapid, and he was able to leave the hospital after 3 weeks. Therapy at home was extended to include, in addition to powdered digitalis 0.1 gm. daily, ammonium chloride 3 days of each week followed by a salyrgan suppository.

When last examined on March 14, 1939, 5 months after his first appearance at the hospital, there were no signs of cardiac decompensation, and on restricted activity he was able to be ambulatory most of the day.

Studies of the Arrhythmia. Of particular interest in this case was the association of cardiac arrhythmia with the phases of the Cheyne-

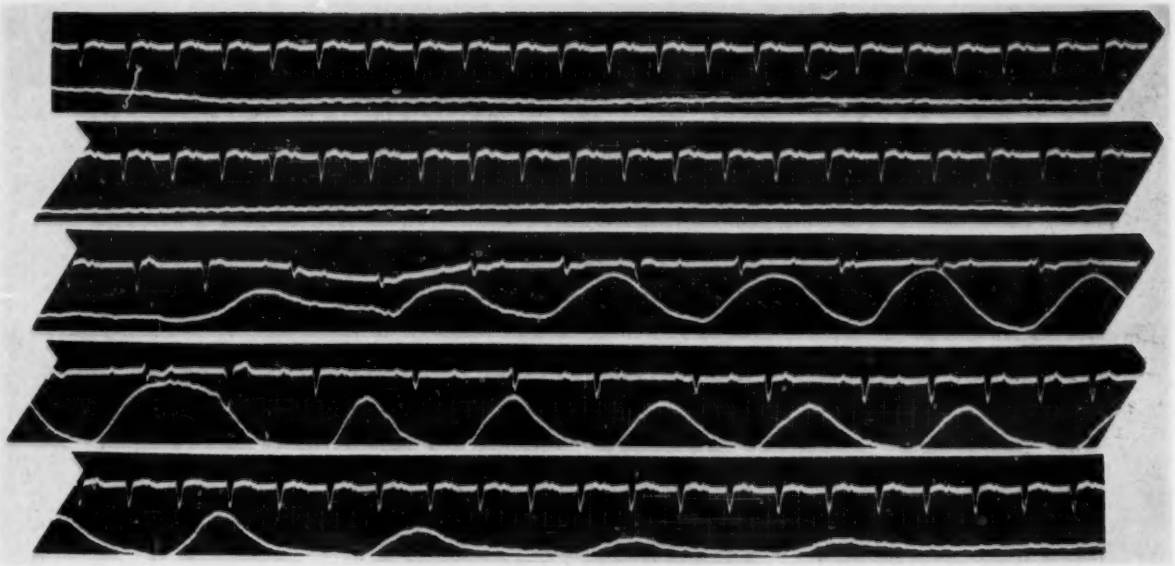

FIG. 1. Case 1. Continuous record through a complete cycle of Cheyne-Stokes breathing, obtained on October 26, 1938. Upper curve, Lead II of the electrocardiogram; lower curve, respiration. In this and in subsequent figures showing respiratory movements, inspiration is recorded as an upstroke.

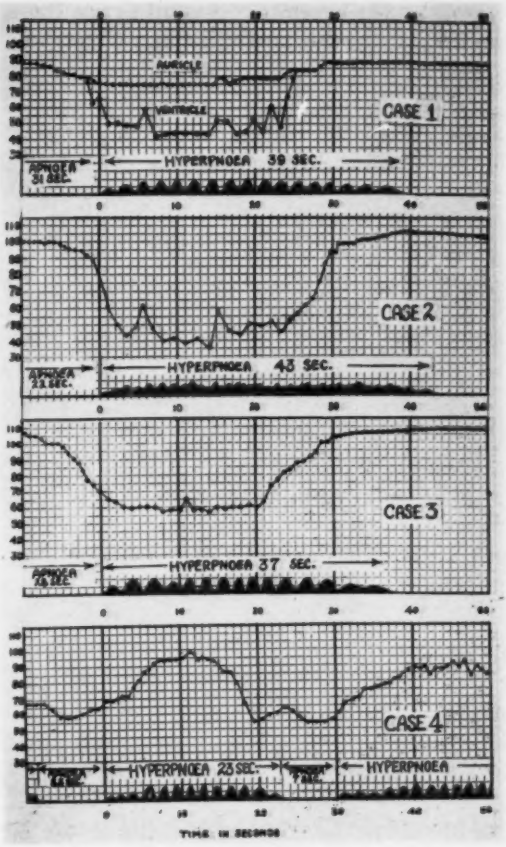

FIG. 2. Chart of the cardiac rate and respiration. The upper curve in each case represents cardiac rate per minute; the black undulations represent respiration. Data obtained from continuous simultaneous records through complete cycles. In the first 3 cases, a part of the cycle preceding the last 10 seconds of apnea is omitted, as the heart rate was constant through this period.

The chart shows that (a) the slowest rate occurred during hyperpnea in Cases 1, 2 and 3, but during apnea in Case 4; (b) in each case a change in rate preceded by several seconds any respiratory movements, and return to former rate occurred before respirations had ceased; (c) in Case 1, the bradycardia was due to complete A-V block, the auricular rate changing but little.

Stokes cycle. The phenomenon was present during the first day after admission and again during the fourth week.

The respirations were recorded simultaneously with Lead II of the electrocardiogram (Figs. 1 and 2). The duration of the Cheyne-Stokes cycle varied from 65 to 85 seconds with 20 to 30 seconds of apnea. During the latter half of hyperpnea and throughout apnea, the cardiac rate was about 90 per minute. Just before the beginning of hyperpnea, there occurred slight sinus slowing, gradual increase in P-R interval to 0.40 seconds and then, rather abruptly, complete auriculo-ventricular block. The idio-ventricular rhythm began immediately without appreciable ventricular asystole. During the period of complete block the auricular rate slowed to 75, the ventricular to about 45, and the form of the ventricular complexes became altered, although not always to the same configuration. On October 4, the ventricular complexes in Lead II during the idio-ventricular rhythm differed from those present through the rest of the cycle in that the QRS was slightly wider, the major deflection was positive and the T-wave negative. On October 26, a different type of ventricular complex appeared during the block, in Lead II the QRS and T-waves being of low amplitude (Fig. 1). At about the middle of hyperpnea the complete A-V block disappeared and the former mechanism returned. The record of the heart sounds revealed a low-pitched diastolic gallop sound present through most of the cycle and disappearing during the period of complete heart block, at which time the high-pitched second sound became widely split.

Following the intravenous injection of 1 mgm. of atropine sulfate, the P-R interval shortened to 0.26 sec. and complete heart block failed to occur during the Cheyne-Stokes cycles of the next few hours. The periodic breathing remained unchanged.

Complete A-V block could be produced on most attempts by pressure over the right carotid sinus and at times by pressure over the left. This block was not spontaneous nor related to phase of respiration since it could be produced during early apnea; and with release of pressure the previous rhythm returned and was followed by the usual spontaneous block at the onset of hyperpnea. The ventricular complexes of the idio-ventricular rhythm produced by carotid sinus pressure were similar to those of the spontaneous block of the same

day (Fig. 3). The facility with which complete A-V block could be produced by carotid sinus pressure seemed to be related to the degree of congestive heart failure rather than to any variations in the P-R interval. Thus, carotid sinus pressure was without effect on March 10, when, although the P-R was 0.32 seconds, signs of cardiac decompensation were absent.

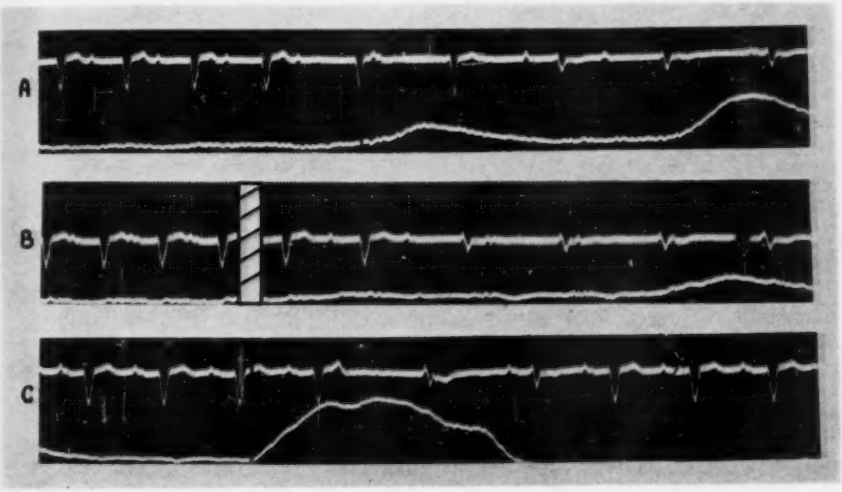

FIG. 3. Case 1. *A*. The spontaneous change in rhythm to complete A-V block at about the onset of hyperpnea.

B. The change in rhythm to complete A-V block provoked during apnea by pressure over the carotid sinus (begun at the hatched area).

C. The change in rhythm produced during apnea by voluntary forced hyperpnea.

The stimuli in *B* and *C* were applied in early apnea so as to avoid the chance occurrence of spontaneous block; that this did not occur is evidenced by the lack of increase in P-R such as preceded the change in rhythm in *A*.

Voluntary forced hyperpnea during early apnea usually had no effect on the cardiac rhythm. On one occasion, however, there resulted a temporary disturbance of the cardiac mechanism with slowing of the ventricular rate, change in the form of the ventricular complexes, probably sinus arrhythmia, and possibly complete A-V block (Fig. 3).

Both the Cheyne-Stokes type of breathing and the cyclic complete A-V block were abolished during the time of inhalation of 5 per cent CO_2 in oxygen, but were not abolished by inhalation of pure oxygen.

On March 10, 1939, the signs of congestive heart failure were absent, the respirations were regular, the P-R interval was 0.32 seconds, and carotid sinus pressure was without effect on the cardiac rhythm. Attempts to produce Cheyne-Stokes respiration by having the patient breathe through a long tube were unsuccessful. The effects of anoxemia with and without hypercapnea were studied. Rebreathing air in a closed system of 7 liters capacity for 5 minutes resulted in an increase in cardiac rate from 53 to 61 per minute. Rebreathing air with the CO_2 being absorbed led to beginning cyanosis in 5 minutes and an increase in cardiac rate from 56 to 74 per minute. In neither instance was there any change in rhythm, in the P-R interval, or in the form of the complexes.

Case 2. H. C.

A 70 year old negress was admitted to The Johns Hopkins Hospital (History No. 155851) November 29, 1938, complaining of shortness of breath and swelling of the legs. She had had a goitre since the age of 20, but her health had been good. She had had one child, and had passed the menopause. She began to have intermittent ankle edema at the age of 61, and at 67 she developed exertional dyspnea and orthopnea, and was admitted to the hospital for 2½ months. The findings at that time were essentially the same as during the second admission. Her recovery was slow and complicated by a peri-rectal abscess requiring incision and drainage. She remained an invalid and had been bedridden for one year when progressive edema and paroxysmal nocturnal dyspnea brought her again to the hospital.

Examination showed drowsiness, obesity, cyanosis, orthopnea, engorgement of the neck veins, and massive edema of the lower part of the body. The respirations were Cheyne-Stokes in type. There was a huge cystic goitre. At both lung bases dulness and moist rales were found. There was generalized arteriosclerosis and the arterial blood pressure was 145/100. The pulse slowed to about half its former rate at the end of apnea. The heart was tremendously enlarged and a presystolic gallop was audible. Abdominal examination was unsatisfactory due to obesity. A small draining sinus was present near the rectum.

Laboratory Examinations. The serological test for syphilis (Eagle) was negative. Examination of the blood was normal. The urine showed 3+ albumin. The non-protein nitrogen of the blood varied from 56 to 37 mgm. per cent; the CO_2 combining power was 48.5 vol. per cent, and the total plasma proteins 6.34 gms. per cent. Phenolsulphonephthalein excretion was 45 per cent in 2 hours.

Roentgenograms showed great enlargement of the heart, tortuosity of the aorta, and several areas of calcium in the enlarged thyroid gland. The electrocardiogram showed normal sinus rhythm, P-R interval 0.16 seconds, QRS 0.08 seconds, left axis deviation, and T-wave changes compatible with digitalization.

Course. After 4 days the Cheyne-Stokes breathing and the cardiac arrhythmia disappeared. With the usual therapy improvement was gradual and she was able to leave the hospital in 2 months' time.

Studies of the Arrhythmia. Simultaneous records of the respiration and Lead II of the electrocardiogram revealed Cheyne-Stokes breathing with apnea of 22 seconds and irregular hyperpnea of 43 seconds (Fig. 2). Shortly before the onset of hyperpnea the cardiac rate

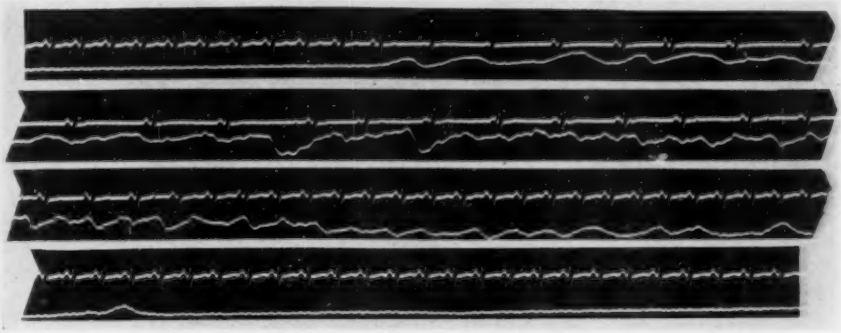

FIG. 4. Case 2. Continuous record through a complete cycle of Cheyne-Stokes breathing. Upper curve, Lead II of the electrocardiogram; lower curve, respiration.

slowed rather suddenly from 100 per minute to about 40; return to the former rate occurred in the latter half of hyperpnea. At the onset of bradycardia the P-waves became lower and the P-R interval shortened; through the rest of bradycardia the P-waves varied slightly in form (Fig. 4). Pressure over the right carotid sinus during early apnea produced similar cardiac slowing with lowered P-waves. Atropine abolished the periodic bradycardia without affecting the Cheyne-Stokes breathing.

Case 3. W. A.

A 38 year old negro laborer was admitted to The Johns Hopkins Hospital (History No. 163619) February 6, 1939, complaining of shortness of breath, cough,

and swelling of the legs. There was an indefinite history of a penile sore 15 or 20 years before, but no specific treatment had been given. He had been able to do hard manual labor until 6 months before admission; since then he had been incapacitated by recurring nocturnal dyspnea, cough, dyspnea on exertion, and swelling of the ankles. He had taken no digitalis.

Examination showed orthopnea, cough, cyanosis, engorgement of the cervical veins, and marked dependent edema. Cheyne-Stokes respiration was present with apnea of 30 seconds. Dulness and moist rales were found at both lung bases. The pulse rate slowed to approximately half its former rate at the end of the period of apnea. The heart was enlarged to the left and the area of aortic dulness was widened. There was a gallop rhythm at the apex and a loud long gushing type of diastolic murmur heard in the second right interspace and down the left sternal border. The peripheral signs of aortic regurgitation were present. The arterial blood pressure was 155/80 during hyperpnea and 170/90 during apnea. The liver edge was palpable 3 fingerbreadths below the costal margin.

Laboratory Examinations. The Wassermann and Eagle tests on the blood serum were positive. No abnormalities were noted in the examination of the blood. On admission the urine showed 3+ albumin; this later disappeared. The non-protein nitrogen of the blood was 45 mgm. per cent, and the CO_2 combining power was 62.6 vol. per cent. Phenolsulphonephthalein excretion was 67 per cent in 2 hours. Roentgenographically there was noted slight dilatation of the aorta with increased pulsation. Electrocardiograms showed sinus rhythm; P-R interval 0.16, QRS 0.08 seconds; normal axis; low T_1 and T_2.

Course. With digitalization, bed rest, and limited fluids, an excellent diuresis resulted and there was a loss in weight of 40 lbs. The Cheyne-Stokes respiration and the cardiac arrhythmia disappeared after the second day. The arterial blood pressure at the time of discharge was 145/55.

Studies of the Arrhythmia. Records of the respiration and of Lead II of the electrocardiogram demonstrated Cheyne-Stokes breathing with apnea of 28 seconds and hyperpnea of 37 seconds (Figs. 2 and 5). Towards the end of apnea, the cardiac rate slowed from 107 to 60 per minute, and this bradycardia persisted till the latter half of hyperpnea, when there was gradual return to the former rate. Sinus rhythm persisted throughout. The P-waves became lower during the bradycardia (Fig. 5).

Case 4. W. G.

A colored male laborer, age 60, was admitted to The Johns Hopkins Hospital (History No. 111587), April 8, 1939, complaining of shortness of breath and coughing up blood. Until 1935 his health had been excellent. At that time he began to have exertional dyspnea and ankle edema, and in 1936 he was admitted

to the hospital in acute left ventricular failure on the basis of hypertensive heart disease. He responded well, but did not follow advice, and in 1937 was admitted a second time in cardiac failure. Again he improved rapidly, but did not continue taking digitalis. Symptoms returned, particularly nocturnal dyspnea, and a third period of hospitalization became necessary.

Examination revealed obesity, orthopnea, cough with reddish frothy sputum, venous engorgement, and slight dependent edema. The respirations at times were Cheyne-Stokes in type. Moist rales were audible throughout both lungs. The peripheral arteries were only moderately thickened. The arterial blood pressure was 180/90. During apnea the pulse slowed to a rate of about 50, and during hyperpnea accelerated to about 90 per minute. The heart was greatly enlarged downward and to the left and there was a presystolic gallop at the apex.

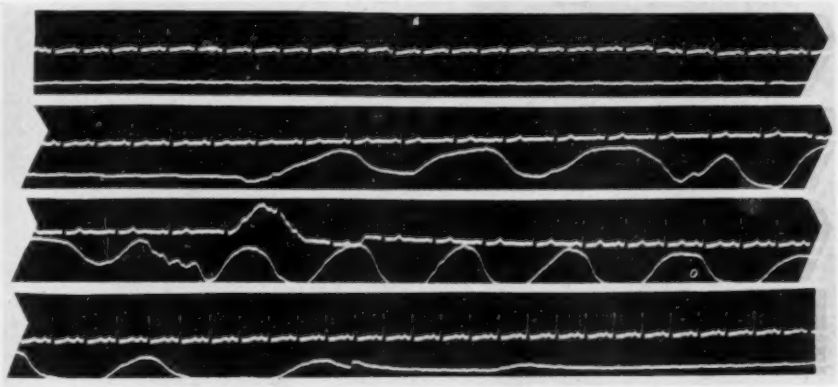

FIG. 5. Case 3. Continuous record through a complete cycle of Cheyne-Stokes breathing. Upper curve, Lead II of the electrocardiogram; lower curve, respiration.

At the base the second aortic sound was loud and tympanitic, and there was a high pitched systolic murmur. The liver edge was just below the costal border.

Laboratory Examinations. The serological test for syphilis (Eagle) was negative. The blood was normal. The urine showed a trace of albumin in the admission specimen, none thereafter; there were numerous leukocytes in the sediment. The non-protein nitrogen of the blood was 32 mgm. per cent. Cultures of the urine were sterile. Phenolsulphonephthalein excretion was 60 per cent in 2 hours. Roentgenograms showed enlargement of the heart and tortuosity of the aorta. Electrocardiograms taken after administration of digitalis revealed left axis deviation, P-R interval 0.20 seconds, QRS 0.10 seconds, T_1 inverted, T_2 biphasic, and T_3 upright. In the chest lead (CF_3) there was a Q-wave followed by a positive major deflection and a negative T-wave.

Course. Digitalis was administered and the signs of cardiac failure rapidly disappeared. Cheyne-Stokes breathing and the cardiac arrhythmia were not present after the first day. The blood pressure at the time of discharge 4 weeks later was 185/100.

Studies of the Arrhythmia. Tracings of the respirations and Lead I of the electrocardiogram showed Cheyne-Stokes breathing with hyperpnea and apnea of variable duration. In figure 2, apnea of 8.5 seconds is followed by hyperpnea of 23 seconds. The cardiac rate changed gradually during the Cheyne-Stokes cycle, reaching a maximum of about 90 per minute in mid-hyperpnea and a minimum of about 55 per minute during mid-apnea. Sinus rhythm persisted throughout (Figs. 2 and 6).

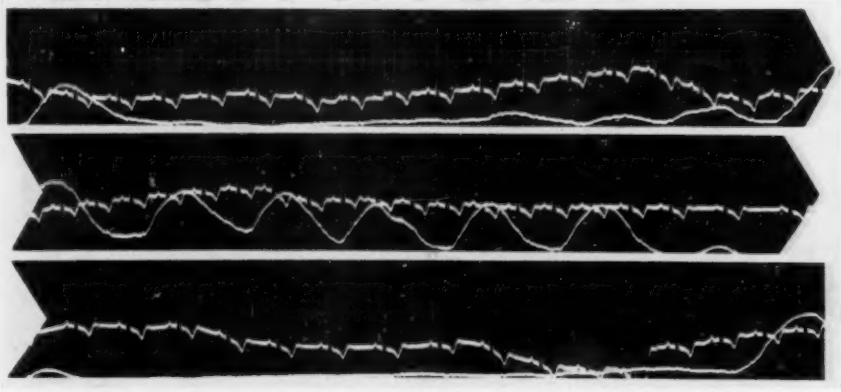

FIG. 6. Case 4. Continuous record through a complete cycle of Cheyne-Stokes breathing. Upper curve, Lead I of the electrocardiogram; lower curve, respiration.

DISCUSSION

There have appeared in the literature numerous reports describing the association of cardiac arrhythmia with the Cheyne-Stokes respiration of cardiac or arterial disease. Of the studies made without the aid of the electrocardiograph (4, 5, 18, 19, 36, 47) only 2 need be mentioned. Baumler's case (4) is of interest because there occurred during hyperpnea cardiac standstill of 30 seconds duration. The

report of Roth (36) is noteworthy since he was the first to demonstrate that atropine abolished the cardiac arrhythmia without affecting the periodic breathing, and that pressure on the neck during apnea produced cardiac slowing similar to that occurring spontaneously during hyperpnea.

The first electrocardiographic study of a patient exhibiting cardiac arrhythmia during Cheyne-Stokes breathing was described by Oppenheimer and Williams (31) in 1913, but the first published electrocardiographic tracings were those of Wasserman (45) in 1922. Since then several reports of single cases have appeared (6, 7, 11, 12, 16, 17, 22, 34, 40, 42, 43). Besides simple sinus slowing, there have been observed various changes in rhythm and mechanism, such as displacement of the pacemaker to the A-V node (34), partial heart block (6, 7, 34, 40), complete heart block (22, 34, 40), and even ventricular standstill of sufficient duration to cause Stokes-Adams attacks (42).[2] In 2 cases where complete A-V block was already present, in one (31) there occurred slowing of the auricle without change in ventricular rate, and in the other (11) slowing of the ventricle without change in auricular rate. In cases where there was ventricular tachycardia during apnea, the rate slowed greatly during hyperpnea (17, 45). During auricular flutter, there was noted in hyperpnea an increase in the degree of A-V block (16, 22). Alteration in the form of the auricular or ventricular complexes has been observed (6, 7, 40, 43), and even disappearance of bundle branch block (12).

In all instances except two the slowing of the pulse and the changes in cardiac mechanism have become manifest during hyperpnea. In one case studied by Biot (5) and in *Case 4* of this report, the slower rate was present in apnea.

Dismissing for the moment these exceptions and considering the phenomenon as it is usually observed, it is evident that the changes in cardiac rhythm and mechanism are those of vagal effect. In all cases where it has been tried atropine has abolished the arrhythmia without altering the periodic nature of the breathing. No satisfactory

[2] This case and the similar one of Baumler (4) are examples of Stokes-Adams attacks secondary to Cheyne-Stokes respirations, and present an interesting contrast to another group of cases in which a type of periodic breathing is secondary to Stokes-Adams attacks occurring in rapid succession (3, 13, 23, 41).

explanation for this cyclic increase in vagal tone has been established, but several possibilities are worthy of consideration.

First, it is possible that the increased vagal tone may be due to reflexes arising from mechanical changes in the lung incident to the act of breathing. Von Saalfeld (44) found in animals that forceful dilatation of lungs provokes a reflex bradycardia. In human subjects, Wilson (48a, b) reported that deep breathing could result in cardiac slowing and other manifestations of increased vagal activity. We observed similar changes produced by voluntary forced hyperpnea (Case 1, Fig. 3). Evidence against this hypothesis, however, is our observation (Figs. 1, 2, 4 and 5) that the manifestations of increasing vagal activity commence several seconds before respiratory movements are discernible, and cease at a time when breathing is still vigorous.

Also to be considered as a possible cause for the cyclic increase in vagal tone is a direct action of the respiratory center on the vagal center, such as has been demonstrated in animals by Anrep *et al.* (1) and by Rijlant (35). Here again, however, in the cases studied it was found that the onset, duration and termination of vagal activity did not parallel the evidence of activity of the respiratory center. It seems unlikely, therefore, that a direct interaction of the centers can explain the phenomenon observed.

Finally, as suggested by Resnik and Lathrop (34), the periodic variations in the chemical composition of the blood may be responsible for changes in vagal activity. Klein (24) and Anthony *et al.* (2) have demonstrated cyclic changes in the concentrations of O_2 and CO_2 in arterial blood during Cheyne-Stokes breathing. Although the magnitude of change was not great, a decrease in O_2 and an increase in CO_2 were found to coincide with an increase in vagal activity (40).

In animal experiments it has been shown that anoxemia first leads to cardiac acceleration, but, if carried to extreme degree, besides having a direct detrimental effect on the conduction system (26, 28, 33), it may cause heart block through vagal stimulation (21b). In normal man, cardiac acceleration is progressive as anoxemia continues to the point of unconsciousness (21a, 38a, b); beyond that stage, in one experiment where the O_2 in the inspired air was 7.2 per cent, there occurred slowing of the pulse and displacement of the cardiac pace-

maker (21a). In recent studies of induced anoxemia in man, May (29) found cardiac acceleration in all of a large series of normal subjects, but Levy *et al.* (25) in 58 observations on cardiac patients, reported 5 instances of bradycardia. It seems reasonable to suppose that anoxemia of less severity might lead to vagal stimulation in the presence of cardiac disease and myocardial failure.

The mechanism by which anoxemia leads to vagal stimulation is not clear. Since the carotid sinus (body) has been designated (8, 20, 37, 39) the chief site of action of anoxemia as a respiratory stimulant, the possibility exists that the carotid sinus may likewise be the site of action of anoxemia as a vagal stimulant. Furthermore, it has been stated (9) that vagal tone is maintained by the activity of aortic, carotid, and other reflexes rather than by impulses arising spontaneously in the vagal center. Although no experimental evidence bearing specifically on this point has been found, there is clinical evidence to suggest that the carotid sinus may play a role in the cardiac arrhythmia associated with Cheyne-Stokes breathing. Most of the case reports have described elderly patients with arteriosclerosis or hypertension, and in this type of subject the carotid sinus reflexes are apt to be particularly active (46). Also, many of the patients had received full doses of digitalis (40) and, as Comeau (7) pointed out, this drug is known to increase the sensitivity of the sinus reflex (15, 30). Furthermore, Roth (36) by pressing on the neck of one of his patients was able to produce, during apnea, a change in cardiac rhythm similar to that occurring spontaneously in hyperpnea. We have been able to repeat this observation in Case 1 (Fig. 3) and Case 2 of this report.

As regards the action of increased arterial CO_2, evidence indicates that its effect on vagal tone is slight, and that its action at the carotid sinus leads to cardiac acceleration through stimulation of the sympathetic centers (9, 27).

There remain to be considered the two cardiac cases mentioned above in which maximum slowing of the pulse occurred during the period of apnea. This behavior of the pulse is similar to that observed by Eyster (14) in cases in which the Cheyne-Stokes breathing was due to increased intracranial pressure. In neither patient was

there clinical evidence of increased intracranial pressure, but no measurements of spinal fluid tension were made. Without these data and without actual determinations of the blood gases, no attempt will be made to explain the arrhythmia in these two cases.

SUMMARY

Four cases of cardiac failure exhibiting marked arrhythmia of the heart during the phases of Cheyne-Stokes respiration were studied. Simultaneous electrocardiographic and respiratory records were obtained through the entire Cheyne-Stokes cycle in each case. In three of the cases, bradycardia began in late apnea and continued till the latter half of hyperpnea; in one, the slowing of the pulse began in the latter half of hyperpnea and persisted till late apnea. In one case, in which the initial P-R interval was 0.32 seconds, the bradycardia was due to complete auriculo-ventricular block. In the other three cases the bradycardia was sinus in type, although in two of the three there were changes in the P-waves, suggesting shift in the pacemaker.

Two of the patients had received no digitalis prior to the occurrence of the phenomenon. In the two cases in which it was tested, atropine abolished the arrhythmia without affecting the periodic breathing. Carotid sinus pressure was applied during apnea in two cases and changes in the cardiac mechanism similar to those occurring spontaneously in hyperpnea were produced. In one case, voluntary hyperpnea during a period of apnea provoked a transient alteration in cardiac mechanism. No studies of the arterial blood gases were made.

The reports of previous similar cases are reviewed and possible explanations of the arrhythmia are discussed.

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HYPERTROPHIA MUSCULORUM VERA (DYSTROPHIA MUSCULORUM HYPERPLASTICA) ASSOCIATED WITH HYPOTHYROIDISM: A CASE STUDY

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INTRODUCTION

Hypertrophy of striated muscle under normal and pathological conditions has long been a subject of study and speculation. A review of the literature discloses much diversification and confusion of clinical types and little data concerning etiology, physiology, or treatment.

Wohl (1831) was among the first to recognize abnormal hypertrophy of the skeletal musculature. Subsequent descriptions of so-called "true" muscular hypertrophy were made by von Stoffella (1863, 1865), Eulenburg (1865, 1875), Auerbach (1871), Krau (1876) and Blum (1879). Krabbe (1921) discussed his own case together with twenty earlier reports many of which were unconvincing or confused with other myopathies. Bruck (1889) first distinguished the congenital form of generalized muscular hypertrophy lately reviewed by Hall et al. (1936). Fulda (1894) contrasted the "true" with the "pseudo" varieties.

Because of the rarity of the disease and lack of investigation concerning it, few studies are conclusive regarding hypertrophia musculorum vera as a clinical entity. Usually it has been classified as a form of myopathy and reported alone or in association with hypothyroidism. The description given by Woods (1911) of a case later reconsidered by Spiller (1913–14) and followed-up by Fay (1922) remains classical. These authors characterized the disease as a rare condition occurring usually in adult males and featuring a long benign course, increased muscular bulk and dull pain or tenderness in the muscles. They noted increased firmness, diminished power and early fatigue in muscular activity. Hypertrophy of individual muscle

fibers was observed but no fatty or fibrous proliferation and no degeneration. Neurological and electrical reactions were normal. No references were made to vasomotor changes or thyroid dysfunction.

CASE ABSTRACT

History. The patient, L. F. (J. H. H., 183,645), a 30 year old unmarried white woman of French descent, was first seen in the out-patient department of the Johns Hopkins Hospital on October 2, 1939 and subsequently brought to the medical ward for study. She was complaining bitterly of early fatigability which accompanied painful enlargement and hardening of the muscles of the arms and legs. This difficulty had come on gradually during the preceding two and a half years and interfered extensively with the patient's ability to work as a waitress.

The first manifestation of the patient's illness began early in 1937 as increasing fatigability of the arms while carrying trays. There was no apparent alteration in strength, and rest was completely restorative. However, in the course of the next several months, aching and actual pain in the arm muscles would occur after moderate use and it was soon evident that these muscles, particularly those of the forearms, were becoming larger and harder than usual. A similar condition, noticed first as fatigue in walking, developed in the leg muscles early in 1939. Within a few months, hardening and enlargement of the leg muscles, especially those of the calves, had occurred, attended by painful "tightening" sensations after activity. In order to carry on even light work or to go about her personal duties, the patient needed frequent brief rest periods which always interrupted the continuity of performance. Within the six months previous to admission the muscular enlargement had produced a strikingly masculine configuration of the limbs. The biceps, for example, became so large the patient could not touch her shoulder with her finger tips, and enlargement of the thenar musculature prevented opposition of thumb and little finger. At no time was there difficulty in relaxing the grip or in starting any muscular activity. Moreover, there were no periods of overwhelming muscular weakness or actual paralysis.

Concurrent with the onset of these symptoms and particularly within the year before admission, the patient began to realize definite lethargy and undue sleepiness. The eyelids became puffy and the eyeballs felt heavy. Speech sounded thick and hoarse. Frequent headaches and occasional dizzy spells occurred. Later, slight deafness was noticed in both ears. The skin over the lower legs became smooth and shiny and the extremities tended to remain cold. The patient gained fifteen pounds in weight.

Various forms of treatment were tried with equivocal results. A brief course of thyroid extract seemed to afford some relief but all symptoms returned after its withdrawal. The patient finally agreed to submit to detailed study in the hospital.

Unfortunately the patient knew little of her family history; she remembered no one with difficulties like her own. Her general health previous to the present

illness had been excellent except for diphtheria without sequelae at the age of twenty. A complete system review disclosed nothing of significance. At no time had there been any skin changes suggestive of dermatomyositis. The menstrual and sexual history was entirely normal. A check of the dietary habits revealed no inadequacies. The patient denied the ingestion of raw meats but admitted the possibility of having eaten undercooked pork at times. Further questioning failed to disclose any symptomatic features of trichinosis. The patient had become well adjusted to her difficulties except for periods of discouragement attending failures in treatment.

Physical examination. Aside from the conspicuous abnormalities in the skeletal musculature, the patient showed little of clinical significance upon routine physical examination. Her temperature, pulse, and respirations ran a normal course and the blood pressure averaged 120 systolic and 80 diastolic. On admission she weighed 122 pounds and was 61 inches tall.

In appearance the patient seemed a normally proportioned and nourished young woman, well-groomed, alert and intelligent, though perhaps somewhat lethargic. Her manner was completely feminine and, except for the bulky muscles, nothing in the body-build or hair distribution suggested virilism. The skin was of usual texture everywhere except over the lower legs where it was smooth and somewhat leathery. The distal portions of the extremities remained cold. The hair of the head seemed dry and coarse and there was distinct puffiness of the eyelids which added to the pudgy but not truly myxedematous facies. There were no other trophic or vasomotor phenomena. The mucous membranes were normal. Glandular enlargement was not present. No skeletal abnormalities were evident clinically. Eye studies revealed only slight bilateral esophoria. The otolaryngologist reported mild bilateral deafness of conduction type and slight thickening and incomplete approximation of the vocal cords. The thyroid gland was not demonstrably abnormal. Examination of the lungs, cardiovascular system, and abdomen was negative. The heart was not enlarged. There was a first degree retroposition of the uterus but the pelvic organs were otherwise normal. The neurological examination disclosed no alterations in motor performance, sensory perception, or reflex activity except for those changes in muscular function noted below. The tendon reflexes were somewhat overactive but not abnormal. There were no abnormalities of sphincter activity.

Muscle data. At the time of admission the patient's skeletal musculature (Figures 1 to 4) exhibited generalized and proportionate hypertrophy, such as might be observed in a male athlete, and in many ways suggested the expression "muscle-bound." Slight enlargement of the facial muscles accentuated the pudgy expression and the masseters seemed larger than normal. The neck muscles were definitely increased in size. Adipose tissue obscured any visible changes in the trunk musculature, but on palpation decided hypertrophy, especially in the shoulder girdle and spinal groups, was evident. Most striking were the symmetrically enlarged muscles of the extremities where there was no excess fat to obscure their size. In the arms the deltoid, biceps brachii, and forearm

muscles stood out conspicuously, especially after use, even though their outlines partly blended with the typical female subcutaneous tissue. The patient could not touch her shoulders with the finger tips. The thenar and hypothenar muscles were markedly hypertrophied, and the enlarged interossei bulged dorsally between the metacarpals. Snapping the fingers and opposition of thumb and little finger were impossible. The muscles of the buttocks and thighs were distinctly larger than normal. The gastrocnemius-soleus groups were especially prominent and seemed to cause foreshortening of the tendo achilles and inability to dorsiflex the ankle fully. Except for these minor limitations in motion, the hypertrophy seemed not to affect freedom of activity, and posture and gait were normal.

The muscles themselves felt much firmer than those of the average woman. They were not stony hard, however, and did not have the doughy consistency typical of myositis nor the woody consistency characteristic of fibrotic muscles. Furthermore, none were actually tender on percussion or deep pressure, and no evidence of local wasting or fasciculation was observed. There was no tremor or incoordination of movement at any time. After use of any particular muscle group, further swelling and hardening would occur, as though the muscles had become engorged. At the same time, the patient would complain of the aching, "tightening" sensations, locally. Frequently she would become exasperated and refuse to continue to use the muscles as requested.

Dynamometry, utilizing a method[1] previously devised to test power in the finger flexors, failed to show any deviation from normal in the immediate strength, work output, or contraction rate. As a matter of fact the values were somewhat above those for the average woman. However, activity prolonged beyond the duration of the test period would initiate such discomfort in the arm muscles that a true estimation of the eventual fatigue rate and working ability could not be made. In general, a brief period of maximal muscular activity, such as lifting, was tolerated more easily than protracted or repetitious mild activity, such as writing or walking.

The myotonic phenomenon was entirely absent after voluntary contraction, muscle percussion, or galvanic stimulation. Furthermore, the hypodermic administration of 2 mgm. of prostigmine failed to cause its appearance; instead,

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[1] This method of dynamometry was employed earlier by Hesser *et al.* (1940) in studying dystrophic patients. It requires only a short test period. By means of the ordinary kilogram dynamometer, the contraction of all the finger flexors was recorded graphically to show strength (single maximal contraction), work output (or working ability), and maximal flexion rate (varying inversely with muscular tonus). Work output was measured by having the subject perform maximal contraction every two seconds for two minutes, the initial and final values affording satisfactory comparative estimation with the intervening strokes creating a fatigue curve. The flexion rate was determined over a fifteen second period of gripping as hard and as fast as possible.

generalized severe muscular twitchings developed and persisted for several hours after injection. These have been considered by Goodman and Bruckner (1937) as evidence of prostigmine intoxication. However, the small dose used in this case would seem rather to indicate specific sensitivity, especially since no other symptoms indicating parasympathetic over-stimulation were evident at the time. Prostigmine administered in comparable doses to an ordinary cretin produced no similar response.

The diminishing tendon-reflex activity of myasthenia and Jolly's sign after protracted faradic stimulation were not obtained. Moreover the administration of quinine in doses sufficient to initiate any latent myasthenic reaction failed to induce it.

No evidence of latent tetany could be demonstrated by nerve percussion, vascular stasis, or hyperventilation.

Muscle tissue (Figures 5 to 8) excised from the forearm extensors disclosed none of the histological features of a true muscular dystrophy. In general, the muscle fibers were normal in size (average diameter 40–60 microns) as well as in distribution, configuration and staining properties. The number, position and arrangement of the sarcolemmal nuclei were not unusual and the longitudinal striae and cross markings of each fiber were intact. No inflammatory reaction or fibrous proliferation was evident and the blood vessels appeared normal. Most striking, however, were the occasional small localized areas of granular degeneration confined to parts of one or more fibers. Sarcolemmal nuclei were present in these regions but the sarcoplasm was replaced by minute granules staining darkly with hematoxylin and resembling calcium deposits. There was no related connective tissue response and the adjacent sarcoplasm showed a hyaline change that quickly merged into the surrounding normal tissue. The reaction in no way suggested that associated with trichina and could be classified only as some non-specific degenerative process. Skeletal muscle taken from a typical cretin showed no similar changes.

Biopsy of the skin revealed tissue which was normal in all respects.

Other clinical laboratory data. The Wassermann reaction on the blood serum was negative.

The patient ran a leucocytosis as high as 15,760 during the first few weeks of hospitalization. Thereafter the white cells were normal in number. However, a constant relative and absolute eosinophilia of about 7 per cent persisted along with a slight increase in lymphocytes. The blood picture otherwise was entirely normal.

Repeated urine examinations disclosed slight albuminuria and moderate pyuria which probably accounted for the leucocytosis. Urine cultures revealed the presence of B. coli.

The stools were negative for occult blood, parasites, and ova. Cultures disclosed no pathogens.

Chemical studies of the blood: Calcium, 10.6 mgm. per 100 cc.; phosphorus,

4.4 to 3.5 mgm. per 100 cc.; protein 8.0 gm. per 100 cc. (Refractive index); and phosphatase activity, 2.6 units. All values are normal.

The creatine-creatinine metabolism was studied on a creatine-free diet. The excretion of creatinine was 1.076 gm. per 24 hours, a somewhat high value. There was no creatinuria. The creatine tolerance test, carried out according to a method described by Thorn (1936), resulted in 92 per cent retention of the 2.64 grams of ingested creatine. This value seems high but is well within normal limits.

Total 24-hour urine specimens were assayed for sex hormone content. Estrogenic substances were present in less than 10 r.u. per 24 hours (10–60 r.u. is normal), and the urinary prolan was less than 14 r.u. per 24 hours (none normally except at ovulation). The androgenic assay, utilizing the castrate rat-colchicine method, disclosed less than 14 i.u. per 24 hours, an entirely normal value. The patient was in the premenstrual period when the urine specimens were obtained.

A roentgenogram of the chest was negative and the heart was of normal size. Further radiographic studies disclosed "long bones of masculine type, but feminine type vertebrae. Hypertrophy and increased density of muscles."

The skin sensitivity test for Trichina spiralis was negative in 1:10,000 concentration but decidedly positive in 1:500.

The basal metabolic rate on admission was minus 28.

FURTHER OBSERVATIONS DURING HOSPITALIZATION

The patient was hospitalized for two months. A constantly low basal metabolic rate and clinical features resembling those of myxedema suggested treatment with thyroid extract. Dosage was regulated to bring the basal metabolism approximately to normal. At the same time an attempt was made to evaluate treatment by means of dynamometry, measurements of muscle size and body weight, circulation studies, and other objective and subjective changes.

For the first week 96 mgm. of thyroid extract were given orally every day with no appreciable effect. This dose was doubled for the next two weeks, whereupon the weight fell from 122 to 112 pounds and the basal metabolic rate rose from minus 28 to plus 10. Finally 128 mgm. of thyroid extract were found to be the optimum daily dose at which this weight and metabolic rate became stabilized. During the last week of hospitalization 10 mgm. of benzedrine sulfate were given with the thyroid extract and accompanied by small postprandial doses of phenobarbital.

Definite subjective improvement accompanied treatment. The patient soon felt more industrious and cheerful. Headaches and dizzy spells were less severe; these responded best to benzedrine sulfate and

phenobarbital. The feeling of fatigability seemed not appreciably altered, but the aching, "tightening" sensations after prolonged muscular activity were less severe though still present. None of the usual toxic effects of thyroid overdosage were noted.

The patient's whole appearance was gradually transformed. The facial expression brightened and the puffiness of the eyelids disappeared. The extremities felt distinctly warmer and more moist and the skin of the legs resumed a more natural texture. Hoarseness and mild deafness continued, however, and the hair remained as before.

TABLE I

	PATIENT (BEFORE TREATMENT)		NORMAL "A"	
Age (years)	30		28	
Height (inches)	61		62.5	
Weight (pounds)	122		115	
Ideal weight (pounds)	105		112	
Neck (cm.)	34.5		30.8	
Arm (cm.)	26.5 (right)	26 (left)	24.5 (right)	24 (left)
Forearm (cm.)	26.5	26.5	22.6	22.3
Calf (cm.)	33.5	34	34.5	34.5
	PATIENT (AFTER TREATMENT)		NORMAL "B"	
Age (years)	30		28	
Height (inches)	61		62	
Weight (pounds)	112		110	
Ideal weight (pounds)	105		110	
Neck (cm.)	33		30.5	
Arm (cm.)	23.5 (right)	23.5 (left)	23.6 (right)	22.5 (left)
Forearm (cm.)	24	24.5	23	22
Calf (cm.)	33	33	33	32.8

Most noteworthy, during the period of treatment with thyroid, was the conspicuous alteration in the skeletal musculature. Although there was some loss of subcutaneous tissue the muscles themselves became definitely smaller (see measurements) and softer, and their tendency to swell and harden after use was diminished. Eventually the patient could snap her fingers, oppose thumb and little finger and touch her shoulders with the finger tips. She seemed better able to perform her personal duties and even carried trays in the hospital dining room with little difficulty.

Circumference measurements made on the patient before and after treatment are given. The corresponding measurements of two normally proportioned women of comparable weight, height and age are offered for contrast (Table I).

The differences in the measurements of patient and normals are too small to be significant. However, it must be remembered that the patient's subcutaneous fat was less and the muscle mass therefore greater than that of the normals. The reduction in the circumference of the patient's neck and limbs after treatment is significant because there was little actual loss in the subcutis.

TABLE II

	PATIENT (BEFORE TREATMENT)		NORMAL "A"	
	Right	Left	Right	Left
Strength (kgm.)	43	43	36	32
Work output (kgm.)	42–28	39–27	36–24	32–23
Maximal flexion rate (15 sec.)	30	31	31	40
	PATIENT (AFTER TREATMENT)		NORMAL "B"	
	Right	Left	Right	Left
Strength (kgm.)	38.5	37	35	32
Work output (kgm.)	36–27	35–25	33–25	32.5–19.5
Maximal flexion rate (15 sec.)	29	30	28	32

After prolonged activity, as in the dynamometric test described for estimating working ability, the patient's forearm musculature, before treatment, increased 1 cm. or more in circumference. After treatment a swelling of only 0.2–0.3 cm. occurred. The forearms of the normal subjects swelled no more than 0.3 cm. after a similar performance.

Dynamometry was performed, by the method described, every 2–3 days during treatment. A comparative study of the values obtained by the patient and those made by normal women of corresponding height, weight and age was impossible because no one could be found with a muscular bulk similar to that of the patient. Thus the patient's measurements approach those of the normal controls described above, but the difference in the muscle-fat ratio prevents a true comparison of dynamometric capacity per unit of muscle mass. In spite

of the larger musculature of the patient, therefore, her dynamometric capacity per unit of muscle mass could be considered equal to or less than that of the controls although her actual capacity, as tested, was distinctly greater.

As judged by the patient's best dynamometric efforts before and after, treatment seemed to cause a slight fall in strength, some tendency to increased fatigability and diminished work output, and no change in contraction rate. The control values are given for the sake of completeness (Table II).

Normal individuals always show slight improvement after repeated use of the dynamometer. Hence the reduction in the patient's values serves further to indicate definite alteration in muscular function associated with treatment.

Studies on the peripheral circulation were conducted by Dr. Robert W. Wilkins in the Department of Medicine. The blood flow to the right forearm and the right calf was measured by means of segment plethysmographs, while the circulation to the hand and foot was occluded by cuffs inflated at 250 mm. Hg. The water in the plethysmographs was maintained at a temperature of 32°C. The patient was kept warm by means of electric heating pads and blankets in a draught-free room at a constant temperature (22°±1°C.).

"Before treatment the rate of blood flow in the right forearm averaged 2 cc. per 100 cc. per minute; the flow in the right calf was 1 cc. per 100 cc. per minute. Normal vasomotor responses were present in these areas: (a) On immersing the left hand in water at 3.2°C. (Hines-Brown cold test), the blood pressure rose from 105/86 to 118/92, and there was a decrease in the blood flow in the right forearm to 1.25 cc. per 100 cc. per minute and in the right calf to 0.75 cc. per 100 cc. per minute. (b) Solving arithmetic caused the blood flow in the right forearm to increase to 6.8 cc. per 100 cc. per minute, and in the right calf it rose to 5 cc. per 100 cc. per minute.

"Exercise by tonic contraction of the hand on a rubber bulb caused the blood flow in the right forearm to increase to 22 cc. per 100 cc. per minute immediately after terminating the exercise. It was impossible to make accurate measurements during the exercise.

"A normal vasomotor response in the fingers and toes was shown by the marked increase in temperature of the finger and toe pads from 24°C. to 34.5°C. when the patient's body was warmed by the electric pads.

"Occluding the circulation to the right forearm and right calf for a period of 5 minutes brought no symptoms of pain or aching, and on release of the circulation

(reactive hyperemia) the blood flow rose to 25 cc. per 100 cc. per minute in the right forearm and to 12 cc. per 100 cc. per minute in the right calf.

"The patient was seen again after treatment and the foregoing studies were repeated. The principal change at this time was a marked increase of the resting blood flow in the right forearm, which now averaged 7 cc. per 100 cc. per minute, and in the right calf, which averaged 4.5 cc. per 100 cc. per minute. Vasomotor responses were present as before.

"Reactive hyperemia produced by occluding the circulation for 5 minutes raised the blood flow in the right forearm to 45 cc. per 100 cc. per minute, and in the right calf to 26 cc. per 100 cc. per minute. It is of interest that the volume of the right forearm (taken at the same distance below the elbow in both experiments) had decreased from 680 cc. to 590 cc. after treatment and that of the right calf (taken at a fixed distance just below the knee) from 1200 cc. to 1110 cc. Such a change is well beyond the limit of experimental error.

"*In summary* it may be said that when the patient was first seen the blood flow to the arm and leg was on the very lower limit of normal for a patient at rest and warm. Measurements after treatment were remarkable because both the resting flows and the reactive hyperemia flows were on the upper border of normal. It is more than likely that this striking change was directly associated with the increase in the basal metabolic rate from minus 28 to plus 10 after thyroid medication."

Except as noted above, the patient's clinical status, as far as observed, was unaltered by treatment. There were no abnormal neurological manifestations at any time, and myotonia, myasthenia, or tetany were never demonstrated. Unfortunately, the eye muscle balance studies were not repeated and reexamination of the ears and vocal cords was omitted. Likewise, failure to follow electrocardiographic changes or to recheck the blood chemistry, creatine tolerance, muscular histology, and prostigmine sensitivity made post-therapeutic observations far from complete. These omissions should serve to guide future studies.

Addenda. The patient left the hospital and stopped taking thyroid extract. In a communication six weeks later, she declared that after working three weeks as a waitress all her old complaints had returned. Most conspicuous were headaches, puffiness of the eyelids, increased hoarseness and great aggravation of the aching "tired" sensations in the arms and legs. She was very anxious to resume treatment.

A second communication, received four weeks after the first, described greater swelling of the face and extremities than ever before. In addition, numbness and pallor of the fingers had developed to-

gether with increased discomfort in the muscles of the legs. All symptoms were greatly aggravated by cold. The patient was still without treatment.

CASE DISCUSSION

Most remarkable for its similarity to the present case is the one reported by Mollaret and Sigwald (1939): an adult male who exhibited generalized muscular hypertrophy together with signs of myxedema. Evidence of hypothyroidism, loss of sex power, and muscle changes had developed concomitantly within a year. Most striking were the large strong muscles which grew weak and painfully hard after any prolonged activity. Muscle biopsy showed slight inequality in the calibre of fibres with some dissociation of fibrils but no sarcolemmal changes or interstitial infiltration. The neurological examination was negative. Eosinophilia of 6.5 per cent was present. X-ray study revealed cardiac enlargement. No investigations were made of sex hormone activity, creatine tolerance, or trichina sensitivity. Treatment with prostigmine was tried first but had to be discontinued because it caused muscular twitchings. After prolonged administration of thyroid extract, however, all the patient's symptoms and signs, both myxedematous and muscular, subsided. Eosinophilia diminished, sex activity was restored, the heart became smaller, the skeletal muscles reverted to normal size, and muscular cramps and fatigability disappeared.

Electrical studies were made on the same patient by Bourguignon (1939). He demonstrated a myotonic type of electromyographic response which, together with certain electrocardiographic abnormalities, returned toward normal with the administration of thyroid extract. He asserted that myotonia could be demonstrated electrically only in certain areas in the patient's muscles (*la petite myotonie*) and was therefore missed clinically.

The authors reviewed the entire subject of muscular hypertrophy. They concluded by affirming a certain control by the thyroid gland over the trophic activities and contractile qualities of the muscle fibre. Moreover, they maintained a strict distinction between the muscle changes due to thyroid dysfunction and those of the true myopathies.

This case report bears much in common with ours as well as with

that of Woods. Certain identical and contrasting features are of particular interest:

Our patient is unique because hypertrophia musculorum vera is particularly rare in women. This fact correlates with the normally smaller muscles of females, a secondary sexual characteristic.

The coexistence of muscular hypertrophy and hypothyroidism is more than coincidental. The former seems to have preceded the latter in onset. Increased muscle bulk might be thought to interfere with a true calculation of the basal metabolic rate, but certain other definite clinical features of myxedema cannot be denied. Further interrelations will be discussed later.

Hypertrophy in the laryngeal muscles probably accounts for the hoarseness. Conceivably, similar changes in the intrinsic ear musculature could cause a slight degree of deafness and alterations in the extra-ocular muscles be held responsible for the esophoria.

The roentgenographic report of "masculine type long bones" is noteworthy. Such changes are known to result from the demand of heavy muscles on their bony support. There was no indication of alteration in calcium metabolism or parathyroid function.

The quantity of creatinine in the urine is proportional to the size of the functioning muscle mass; hence the heavy creatininuria in our case. According to Thorn (1936), a consistently high retention of ingested creatine seems to be a feature of the hypothyroid state. However, he administered only 2.64 gm. of creatine, nearly all of which most normal individuals will excrete in the 24-hour test period. It is likely that the greater retention of creatine in hypothyroidism could be demonstrated more decisively if larger doses were administered. No data are available in this regard. Of significance is the fact that in restoring the low basal metabolism of myxedema to normal by thyroid administration, the most severe degree of creatinuria occurs early in treatment, an indication of initially high creatine storage.

Little can be said for the changes noted in the muscle biopsy. They compare with neither of those described by Mollaret or Woods. The degenerated areas suggesting calcium deposition might account in part for the muscular discomfort. No similar picture has been reported in other myopathies or hypothyroid states.

The positive trichina skin test is definitive with eosinophilia as evidence supporting an active muscular infection. In 98 to 100 per cent of cases it indicates that the individual's muscles at some time have been invaded by Trichina spiralis. Cross-sensitivity is known to occur rarely with the whip-worm, Trichuris trichiura. Since the degree of sensitivity is roughly proportional to the recency of the infection, our patient's response probably signifies a remote invasion. Muscular swelling incident to immediate extensive involvement has been observed infrequently, yet trichinosis could hardly be considered the cause of the muscular symptomatology and hypertrophy in our case. Moreover, no trichinae were demonstrated by biopsy.

Eosinophilia occurred likewise in Mollaret's patient who might also have had trichinosis. Lack of data elsewhere prevents its being considered a characteristic feature of hypertrophia musculorum vera. Vasilescu (1938) noted eosinophilia in various forms of myositis. He demonstrated none in muscular dystrophies, myotonias, or amyotrophic lateral sclerosis.

Of particular significance is the sensitivity to prostigmine, noted in both cases, as evidenced by muscular twitching after therapeutic dosage. Since prostigmine apparently affects skeletal muscle in part directly, the hyperreactivity of these patients indicates some intrinsic and possibly typical physiochemical alteration in the contractile substance. It is likely that the process is purely local in origin. Evidently it is not related to the hypothyroid state alone. Ford (1939) has designated similar local fibrillary activity as "myokymia."

Ravin (1939) presented an excellent review of myotonia. The latter has been reported in association with various types of myxedema, or as myotonia atrophica or Thomsen's disease associated with hypothyroidism. Prostigmine prolongs contraction in myotonia and increases mechanical irritability. Bourguignon (1939) considered "*la petite myotonie*" of Mollaret's case as myotonia of isolated muscle fibres. Under these circumstances the myotonic response, accentuated by prostigmine, might occur as a progressive transfer of mechanical stimulation from one contracting myotonic fibre to the next and resemble fasciculation. Because of the preponderance of unaffected fibres, the myotonic response *in toto* was not produced clinically.

The blood flow studies are interesting not only in demonstrating the

influence of thyroid on the blood flow in muscle but in suggesting intermittent ischemia as a cause for the muscular discomfort. Our patient's muscles may have enlarged out of proportion to their originally adequate blood supply and become painful after exercise in the manner of intermittent claudication. Diminution in muscle size, increase in blood flow, both relative and absolute, and relief from symptoms occurred when thyroid was administered.

Diminished muscular power after treatment is significant. Normal individuals usually improve with practice at dynamometry. Conditions in our study were ideal because the patient felt better and was anxious to demonstrate her prowess. Therefore the change must reflect the decreased size of her muscles and the exhausting effect of thyroid, which was more pronounced in the presence of a myopathy.

In retrospect, the structural basis for muscular hypertrophy and its reduction after treatment in our case is not entirely clear. Enlargement of the muscles might have been caused by vascular stasis and water retention; and the water might have been released by the accelerating effect of thyroid. This would explain the relief from swelling and pain following exercise as well as changes in muscle measurements and blood flow. However, since the individual fibers were normal in size and showed no edema microscopically, hypertrophy might have been due in part to increased numbers of muscle fibers which diminished in size individually under treatment.

GENERAL CONSIDERATIONS

Further analysis of the cardinal features in this case study leads to the consideration of various types of muscular hypertrophy and their interrelationships. Physiological and pathological varieties are distinguished but are not always clearly differentiated. Characteristic changes in muscular structure and metabolism and the frequently associated endocrine disturbances create striking analogies not only between kinds of physiological hypertrophy but with the various myopathies as well. Lorenz (1904) has made an excellent survey of muscular abnormalities.

Skeletal muscle is a highly specialized body tissue whose structure and function are determined by numerous delicately balanced variables. These include constitutional inheritance, sex, diet and exer-

cise, in addition to the factors of general and muscular metabolism as mediated by circulatory adequacy, nervous control, and the synergistic effect of hormones. Normal muscles may therefore differ widely in form and structure, power and working capacity, efficiency of contractility, and ability to cope with usage. When disease or maladjustment occurs in, or interferes with, one or more of the controlling variables, definite abnormalities result one of which may be hypertrophy. This develops through an increase in the number and/or size of the muscle fibers, or by proliferation of interstitial components.

Under given conditions of diet and exercise certain normal individuals develop muscles much larger than the average. Likewise, local physiological hypertrophy results from over-use of a part, as in the occupational hypertrophies discussed by Grosse (1898), or in poliomyelitis when a normal muscle group is called upon to serve where others have failed. Papanicolaou and Falk (1938) produced muscular hypertrophy experimentally in female and castrated male guinea pigs with testosterone propionate.

Pathological hypertrophy of muscles is found as a primary abnormality or in connection with disease elsewhere. The former includes hypertrophia musculorum vera as well as the congenital, the dystrophic "pseudo" and the Thomsen's myotonic varieties. Secondary muscular enlargement may result from an increase in the fibrous or fatty interstitial tissue, vascular stasis, or infectious, parasitic, or neoplastic infiltration. Sex hormone disturbances create the large musculature of pubertas praecox and virilism.

The association of thyroid dysfunction with abnormalities in skeletal and cardiac musculature and with clinically recognized myopathies is noteworthy. Frequent attempts at etiological correlation have been made. Nothmann (1937) mentions muscle changes in his discussion of thyroid disorders.

Few significant data are available concerning the effect of thyroid hypofunction on striated muscle. Alterations sometimes resembling hypertrophy in the skeletal and cardiac musculature of cretins are recognized. In congenital and post-operative myxedema Slauck (1921) demonstrated peculiar concentric fibrosis of muscle bundles but saw no hypertrophy or degeneration. Although similar changes were

described by Heidenhain in myotonia atrophica, Slauck found none in his series of muscular dystrophies. Weitz (1931) reported the occurrence of a myotonic-like reaction and painful local swelling of muscles after percussion in a case of myxedema following x-radiation of the thyroid gland.

Of interest is the fact that hypothyroidism occasionally coexists with muscular hypertrophy and certain myopathies. Amyot (1938) described a case of myotonia atrophica with severe hypothyroidism. Garcin *et al.*, Bourguignon and Garcin, and Garcin and Bertrand (1935) studied a case of myotonia congenita (Thomsen's disease) which showed muscular hypertrophy and clinical myxedema. They proposed further investigation of muscular structure and function in congenital and acquired myxedema. Poncher and Woodward (1936) discussed the relation of thyroid administration to creatinuria, decreased muscle glycogen and diminished myotonia in an infant with Thomsen's disease. They suggested that the greater ease with which glycogen is utilized in a patient under treatment with thyroid causes muscular relaxation and recovery to be more prompt. Denoyelle *et al.* (1938) and many others have reported congenital muscular hypertrophy associated with myxedema. Some claimed this condition responded remarkably to thyroid treatment. Mollaret's case and ours represent hypothyroidism in relation to hypertrophia musculorum vera.

In contrast to the foregoing concepts, the connection between hyperthyroidism, muscular atrophy and certain other myopathies deserves attention. Ayer *et al.* (1934) presented a case of exophthalmic goitre which also exhibited features resembling progressive muscular atrophy. Muscular twitchings were diminished by treatment with Lugol's solution and apparent restoration to normal occurred after thyroidectomy. A similar case was reported by Starling *et al.* (1938) who included a commentary by W. R. Brain enumerating various muscular disorders known to occur with hyperthyroidism. These were 1) exophthalmic ophthalmoplegia, 2) the thyrotoxic myopathies, including acute bulbar palsy, progressive muscular atrophy and periodic paralysis, and 3) myasthenia gravis.

Further evidence of the thyroid factor in the physiochemical organization of striated muscle was given by Thorn (1936) who reviewed

creatine studies in thyroid disorders. Creatinuria and decreased tolerance to administered creatine occurs in Graves' disease and can be produced by thyroid feeding. Richardson and Shorr (1934) suggested a disorder in the resynthesis of creatine to phosphocreatine. Schauf (1938), however, felt that the administration of thyroxine, glycine, or adrenalin, or the presence of hunger call for an increased breakdown of muscle glycogen which then impairs creatine metabolism.

Eriksson and Petrén (1937) created hypo and hyperthyroid states in guinea pigs and studied the number of patent capillaries per unit volume in the skeletal and cardiac musculature. They concluded that hyperthyroidism greatly increased the capillary density in both types of muscle; they failed to mention structural or chemical alterations in the muscle itself. Their findings may be correlated with the blood flow changes noted in the present case study. Both observations probably represent a reflex vasomotor control of blood flow to a muscle by metabolic changes in the muscle itself. It must be remembered, however, that the loss of muscle substance in the hyperthyroid state might account for a relatively increased vascularity per unit muscle volume. Increased vascularity in muscle might contribute further to the metabolic disturbance of thyroid intoxication.

In considering vascular aberrations, attention must go to the role of muscular respiratory pigment in any disorder of muscle function. Meldolesi (1937) considered disturbances of myoglobin activity characteristic of muscular dystrophies. The properties of muscle pigment were investigated by Millikan (1936, 1937) who showed myoglobin to have from 3 to 15 times more affinity for oxygen than hemoglobin. This is the fastest combining action involving biological substances yet known and accounts for the ready transfer of oxygen from blood to muscle. Interference with this mechanism could arise from conditions affecting blood flow or impairing the function of myoglobin and would result in grave disturbances of contractility and eventually of muscular structure.

Tower (1939) considered occupational atrophy, as well as the atrophy of denervation fasciculation or tenotomy contracture, to be a result of overactivity and exhaustion of the muscle unit. Since hyperthyroidism accelerates metabolism generally and so demands

increased skeletal muscle activity while interfering with its metabolic adjustment, a similar set of circumstances and analogous structural changes may be expected to obtain.

Hines and Knowlton (1933–34) indicated the influence of thyroid on striated muscle cells by demonstrating an accelerating effect of thyroxine on the rate of atrophy in denervated skeletal muscle. In this case it would seem that the metabolic processes incident to denervation were speeded by thyroxine. Furthermore, the muscular contractile mechanism was intoxicated by thyroid while an increased demand was made upon it by the fasciculation overactivity of denervation. Under such circumstances exhaustion and muscular damage would ensue more quickly. Conversely, denervation atrophy should be slowed by thyroidectomy which might retard the progress of any muscle disease, particularly progressive muscular dystrophy.

Pseudohypertrophy, which sometimes occurs in progressive muscular dystrophy, is characterized by swelling and later degeneration of certain muscle fibers. This may represent a train of circumstances whereby the first attempt to make up for the weakness in the dystrophic fibers is hypertrophy in the remaining more normal ones. These later undergo the same dystrophic changes themselves or break down under the strain.

It becomes evident that structural damage to striated muscle can result from any relative or absolute inadequacy in the intrinsic metabolic contractile mechanism. When the supply fails to meet the demand set by muscular activity, exhaustion and atrophy follow at a rate determined largely by the thyroid factor. Whether the reverse may obtain in true hypertrophy of striated muscle remains for speculation. It is conceivable that if overactivity is not too great, the stimulation derived from periodic exhaustion of muscle would cause an intermittently increased glycogen storage, damming of contractile substance in excess of the actual need and eventually muscular enlargement. This sequence should be encouraged by an excess of androgenic hormone, shown by Kenyon *et al.* (1938) to cause retention of electrolytes, nitrogen and water; by the hypothyroid state, where increased creatine retention and vascular stasis seem to occur; and particularly, as suggested by Krabbe (1921), in certain individuals

whose reparative processes tend to be excessive. In the latter case, an analogy was drawn to similar specific tendencies in keloid formation or hypertrophic osteitis. Babinski (1904) had felt that repair in muscle following neuritis might be exuberant in certain cases and lead to hypertrophy. He pointed out the frequency with which typhoid fever had preceded the onset of hypertrophia musculorum vera (diphtheria in our case!).

In any event, therefore, it seems that when the other variables are in order, given conditions of muscular size and activity must be matched quantitatively by an appropriate thyroid factor before function can be achieved properly. This regulation is undoubtedly mediated by the thyrotropic influence of the pituitary. Consequently, unbalanced and possibly abnormal thyroid function may result from any increased demand made upon this regulatory system. Athletes, for instance, frequently have unusually low basal metabolic rates. Likewise, a call for readjustment made by striated muscles which are generally enlarged for any other reason (certain dystrophies and hypertrophia musculorum vera) could influence thyroid function in a similar manner. How this occurs remains unknown. Analogy may be made to so-called "pituitary diabetes" in which it is thought that some unusual demand stimulates a pancreatropic factor of the anterior pituitary to cause exhaustion of the Langerhans cells with hydropic degeneration and eventually permanent hypoinsulinism. Recently Paulson (1939) has demonstrated a direct influence of the thyrotropic factor of the pituitary by producing definite degeneration of skeletal and cardiac muscle in normal and thyroidectomized guinea pigs with injections of "antuitrin T."

The development of a hypothyroid state in this case not only produced its own symptomatology but aggravated that of hypertrophia musculorum vera. Treatment with thyroid extract apparently relieved the symptoms attributable to thyroid dysfunction but not those of the muscular disease itself, whatever its nature. The patient's muscles became smaller just as normal muscles would under parallel circumstances. Theoretically, if there had been a suitable autogenous thyroid response to the disease, muscular hypertrophy would have been less conspicuous or, in a woman, possibly not apparent.

SUMMARY

1. The history, clinical picture and certain investigative aspects of a patient with hypertrophia musculorum vera associated with hypothyroidism are presented.

2. A brief historical review of the disease entity is given.

3. The features of this case are compared with those of a similar case recently reported.

4. An endeavor is made to analyze the studies conducted. Particular attention is paid to muscular alterations noted before and after thyroid administration. Evaluation is given to creatine tolerance, trichina skin testing, eosinophilia, prostigmine sensitivity, biopsy findings, and dynamometric and blood flow changes.

5. General considerations are advanced regarding types of muscular hypertrophy and their interrelationships.

6. The association of thyroid dysfunction with definite abnormalities of striated muscle is reviewed. A survey is made correlating levels of thyroid function, alterations in muscular structure and certain myopathies.

7. Other evidence substantiating the thyroid factor in the physiochemical organization of striated muscle is presented. Its role in creatine metabolism, muscular vascularity, myoglobin function and muscular exhaustion is indicated.

8. In the light of the data presented, an attempt is made to elucidate the cause of muscular hypertrophy, to speculate upon the reason for the associated hypothyroid state and to interpret the immediate results of treatment with thyroid extract.

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PLATE I

FIG. 1. The pudgy features, thick neck and enlarged arm musculature are evident.

FIG. 2. Conspicuous enlargement of trapezius, deltoids, and back musculature.

FIG. 3. Masculine appearance of hands and forearms. The bulging thenar eminence is plainly seen. Tape marks were left from vascular flow studies. Dressing on left forearm marks site of biopsy.

FIG. 4. Enlargement and accentuated contour of calf musculature. The patient was trying to dorsiflex the ankle.

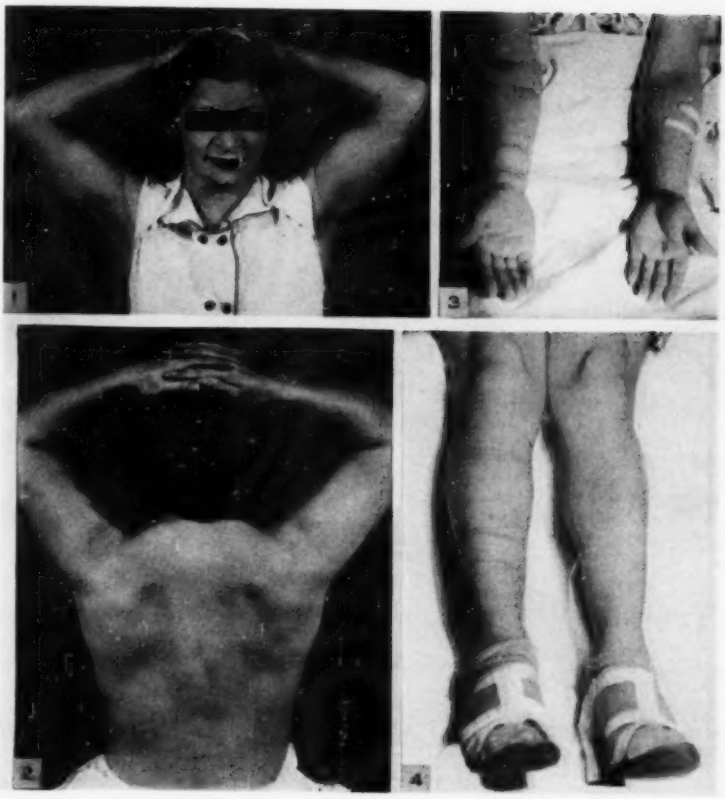
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PLATE II

FIG. 5. Low power photomicrograph (75 diameters) showing generally normal distribution of muscle fibers and sarcolemmal nuclei in longitudinal section. The area of degeneration is visible in the center.

FIG. 6. Oil immersion photomicrograph (345 diameters) of the degenerate area visible in Figure 5. The sarcoplasm is retracted and has a darkly staining granular appearance. Persistent sarcolemmal nuclei and hyaline appearance of the adjacent sarcoplasm in the same fiber. A normal fiber lies along side.

FIG. 7. Low power photomicrograph (75 diameters) of another area in the same biopsy specimen, cut obliquely. The normal appearance of fibers, nuclei, and interstitial tissue is again evident. A second focus of degeneration is seen.

FIG. 8. High dry photomicrograph (133 diameters) of same degenerate area seen in Figure 7. The granular changes resemble those in Figure 6. Note close approximation of normal fibers and absence of inflammatory or fibrous reaction.

UNIV. OF MICH.

FACTORS INFLUENCING PLASMA PROTHROMBIN IN THE NEWBORN INFANT

III. A Study of the Vitamin K Activity of Various Naphthohydroquinone Derivatives

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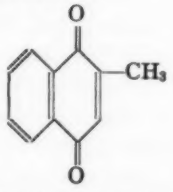
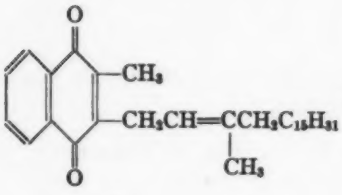
The rapid development of accurate knowledge concerning the chemical structures and synthesis of vitamin K is now one of the classics of modern research. Work on the isolation of the vitamin was initiated independently by Almquist (1–2) and Dam (7) in 1936. Great progress was made by McKee, *et al.* (10), in St. Louis, in 1939, with the announcement of $C_{32}H_{46-48}O_2$ and $C_{40}H_{54-56}O_2$ as the tentative formulas of vitamins K_1 and K_2, respectively. More important than the formulas was the suggestion that the vitamins were quinones. The synthesis of 2-methyl-3-phytyl-1,4-naphthoquinone (vitamin K_1) was announced by Binkley, *et al.* (6), on August 21, 1939, and independently by Fieser (8–9) four days later. The vitamin K activity of the simpler quinones was established by Almquist (3) in studies on phthiocol. Particularly interesting was the announcement of Ansbacher and Fernholz (4) that 2-methyl-1,4-naphthoquinone possessed three to four times the vitamin K activity of the naturally occurring substance. It is the object of this investigation to study the comparative ability of various naphthohydroquinone derivatives in raising the plasma prothrombin levels of newborn infants when fed to their mothers during labor. For this purpose the following compounds have been studied.

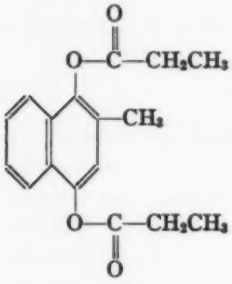


2-methyl-1,4-naphthoquinone

[1] Fellow, National Committee on Maternal Haelth.

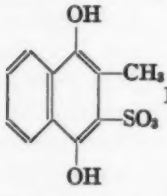


2-methyl-3-phytyl-1,4-naphthoquinone



2-methyl-1,4-naphthohydroquinone dipropionate

The foregoing compounds were dissolved in oil and given by mouth. In addition 2-methyl-1,4-naphthoquinone was made into tablets having a milk sugar base.



2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate

This substance was prepared and studied by Moore, Dann, and Kohn-Richards (11), who have submitted the foregoing tentative formula. It is soluble in isotonic solution and was given parenterally in the following experiments.

The compounds were all administered in unit[2] equivalent doses to the mother during labor. Previous experience had shown that 2,000 units of the concentrate was approximately the minimal effective dose. With this in mind it seemed that the administration of 2,000-unit doses furnished an adequate method of comparing the ability of these various substances to elevate the infant plasma prothrombin level when fed to the mother during labor. As in our earlier studies,

[2] The Ansbacher unit (14) is used throughout.

the method of Smith, Warner, and Brinkhous (13) has been used to determine the plasma prothrombin level.

TABLE I

The plasma prothrombin levels of mother and child, expressed in dilution units, in thirty-one full term deliveries

NAME	RACE	INFANT WEIGHT	PROTHROMBIN, MOTHER	DILUTION UNITS, INFANT
C. B.	W	3,660	138.9	39.2
Z. B.	C	2,825	202.9	13.7
R. B.	W	4,010	201.2	33.8
E. B.*	W	2,880 2,800	92.4	28.2 26.8
N. B.	C	3,305	157.0	41.3
N. B.	C	3,600	45.0	14.0
M. C.	C	4,075	99.0	36.5
M. C.	W	3,120	45.8	14.8
N. E.	W	3,290	130.6	26.8
B. F.	C	2,465	237.6	60.4
M. G.	C	2,535	173.2	32.2
J. G.	W	3,620	129.8	15.0
E. H.	W	3,550	124.4	25.6
B. H.	W	3,800	150.5	28.6
A. I.	W	2,360	149.9	32.6
E. J.	C	3,040	151.8	34.4
D. J.	C	3,000	85.0	14.9
M. J.	W	3,850	295.4	36.0
R. K.	W	3,310	129.8	62.5
S. M.	C	3,180	61.6	21.3
E. R.	W	3,920	85.8	20.0
E. S.	W	2,670	123.8	22.4
M. S.	W	3,110	144.0	34.4
C. S.	C	2,760	117.0	28.4
B. T.	C	2,675	46.2	14.2
M. T.	W	3,700	189.0	33.7
A. W.	C	2,675	99.8	33.8
P. W.	C	3,775	79.2	20.5
R. W.	C	3,010	164.3	26.6
E. W.	C	2,470	124.4	27.7
G. Z.	W	3,400	97.4	13.8
Average		3,201.2	131.4	28.6

* Double ovum twins.

Table I presents the data in the control group, and is taken from a previous publication in this series (12).

In Table II the plasma prothrombin levels of mother and child in twelve deliveries are recorded. These mothers were fed 2,000 units of vitamin K concentrate in oil during labor. In Table IX the statistical analysis of the results is given. While the concentrate in this dosage was able to raise the mean level of the infant plasma prothrombin, it will be seen that the ratio of the standard deviation of the difference of the means to the difference of the means is only 1.4 and, therefore, not significant. This is the result that could have been

TABLE II

The plasma prothrombin levels of mother and child, expressed in dilution units, in twelve deliveries following treatment of the mothers with 2,000 units of vitamin K concentrate during labor

NAME	INFANT WEIGHT	HOURS FROM ONSET OF TREATMENT TO DELIVERY		PROTHROMBIN DILUTION UNITS		
				Mother prior to treatment	Mother at delivery	Infant at delivery
		hours	*minutes*			
L. R.	2,965	10	02	178.6	206.3	55.0
C. S.	3,200	8	06	211.6	244.2	70.4
H. L.	3,750	7	37	127.6	141.5	28.8
T. C.	3,380	7	35	191.4	222.7	33.0
C. C.	3,250	5	26	123.2	132.1	26.0
M. J.	3,725	4	30	136.4	156.0	54.6
A. W.	3,510	4	10	195.3	216.5	34.7
M. K.	3,620	2	40	99.0	112.0	20.6
E. C.	3,360	2	25	190.0	221.6	72.6
H. C.	3,160	1	47	120.2	129.9	00.0
A. G.	3,270	1	45	215.3	229.5	35.8
A. H.	3,140		48	210.5	217.6	48.1
Average				166.5	185.8	39.9

expected on a theoretical basis; in other words, an effective drug (12) has been given in doses so small that only a portion of the recipients were affected.

Table III records ten deliveries in which the mothers received 2,000 units of 2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate intramuscularly during labor. As demonstrated in Table IX, there is a slight but insignificant elevation of the mean infant plasma prothrombin level.

TABLE III

*The plasma prothrombin levels of mother and child, expressed in dilution units, in ten deliveries following treatment of the mothers with 0.5 cc. of 2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate, intramuscularly, during labor**

NAME	INFANT WEIGHT	HOURS FROM ONSET OF TREATMENT TO DELIVERY		PROTHROMBIN DILUTION UNITS		
				Mother prior to treatment	Mother at delivery	Infant at delivery
		hours	*minutes*			
J. E.	3,180	20	30	109.4	121.6	35.8
M. C.	3,715	13	55	99.0	105.0	21.5
R. R.	2,375	10	35	112.2	112.2	21.4
A. K.	3,150	10	15	114.8	142.7	40.2
M. H.	3,040	9	51	131.6	138.0	33.5
L. M.	2,510	8	23	57.9	89.3	27.7
R. C.	3,430	6	30	102.9	102.9	29.2
G. G.	3.705	2	46	102.9	140.3	68.7
T. T.	3,630	2	10	109.3	108.4	21.9
E. C.	3,500	1	20	135.1	141.5	27.7
Average	3,223			107.5	120.1	32.7

* 0.5 cc. 2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate is equivalent to one milligram of 2-methyl-1,4-naphthoquinone.

TABLE IV

*The plasma prothrombin levels of mother and child, expressed in dilution units, in twelve deliveries following treatment of the mothers with one milligram of 2-methyl-1,4-naphthoquinone during labor**

NAME	INFANT WEIGHT	HOURS FROM ONSET OF TREATMENT TO DELIVERY		PROTHROMBIN DILUTION UNITS		
				Mother prior to treatment	Mother at delivery	Infant at delivery
		hours	*minutes*			
G. R.	1,720	11	50	102.5	132.1	38.4
E. M.	3,925	10	50	125.4	124.3	27.0
F. D.	3,910	10	35	125.4	145.2	35.4
L. W.	3,500	7	40	120.7	140.4	42.6
B. D.	3,055	5	25	116.8	143.6	33.6
I. L.	3,060	4	30	148.8	161.2	27.8
G. H.	4,060	4	01	118.0	138.5	39.1
L. W.	2,100	3	18	84.5	104.0	36.5
H. R.	3,180	2	37	103.5	125.3	38.4
G. B.	2,850	1	15	109.4	128.7	47.0
I. C.	3,250	1	10	114.0	126.3	24.8
H. B.	3,340	1		124.4	136.4	32.5
Average	3,350			115.1	133.8	35.2

* One milligram made into milk-sugar pills (one milligram equals 2,000 units).

Table IV records twelve deliveries in which the mothers were given 2-methyl-1,4-naphthoquinone in 2,000 unit milk-sugar pills during labor. Again Table IX demonstrates that the slight rise in the mean infant plasma prothrombin level is insignificant. While this substance

TABLE V

*The plasma prothrombin levels of mother and child, expressed in dilution units, in seventeen deliveries following treatment of the mothers with two milligrams of 2-methyl-1,4-naphthohydroquinone dipropionate during labor**

NAME	INFANT WEIGHT	HOURS FROM ONSET OF TREATMENT TO DELIVERY		PROTHROMBIN DILUTION UNITS		
				Mother prior to treatment	Mother at delivery	Infant at delivery
		hours	*minutes*			
V. K.	3,060	19	05	105.6	121.5	41.3
M. T.	2,800	12	50	104.7	132.0	36.8
E. G.	2,700	12	33	121.2	146.7	36.0
M. D.	3,360	12	30	139.2	159.5	35.8
F. W.	3,610	11	15	96.0	115.2	39.6
G. G.	3,340	8	05	110.5	153.3	41.6
J. W.	4,110	8		142.7	163.6	40.2
E. F.	3,675	6	30	108.5	127.6	32.6
B. B.	3,400	4	54	134.4	148.0	35.8
M. K.	2,800	4	10	125.4	150.5	49.7
M. B.	3,130	3	19	121.6	143.0	39.6
B. K.	2,970	3		107.5	121.2	35.9
V. B.	3,000	2	40	129.6	155.3	43.7
D. L.	3,240	2	30	122.3	148.0	33.6
G. J.	3,000	2	28	120.2	140.4	32.5
N. J.	2,270	2	06	117.0	126.5	46.6
E. S.	3,570	2	02	127.6	148.0	46.5
Average	3,178.5			119.6	141.1	39.2

* Two milligrams of 2-methyl-1,4-naphthohydroquinone dipropionate equal 2,000 units.

is effective in this dose when dissolved in oil, in all probability the milk sugar pill was so insoluble that no elevation occurred.

Seventeen mothers were given 2,000 units of 2-methyl-1,4-naphthohydroquinone dipropionate during labor, as recorded in Table V. The mean infant plasma prothrombin level was elevated to 39.2 units. That this is a significant difference from the control group is shown in Table IX.

Table VI records the plasma prothrombin level of mother and child in ten deliveries, the mothers having been given 2,000 units of 2-methyl-3-phytyl-1,4-naphthoquinone during labor. The mean plasma prothrombin level of the newborn infants was 38.6. The significance of this elevation is demonstrated in the table on statistical analysis.

2-methyl-1,4-naphthoquinone, in 2,000 unit doses, was given to twenty-seven mothers in labor. The mean infant plasma prothrombin

TABLE VI

*The plasma prothrombin levels in mother and child, expressed in dilution units, in ten deliveries following treatment of the mothers with four milligrams of 2-methyl-3-phytyl-1,4-naphthoquinone**

NAME	INFANT WEIGHT	HOURS FROM ONSET OF TREATMENT TO DELIVERY		PROTHROMBIN DILUTION UNITS		
				Mother prior to treatment	Mother at delivery	Infant at delivery
		hours	*minutes*			
R. F.	2,930	28	47	136.3	136.3	26.4
M. J.	3,800	17	17	115.3	138.6	44.6
C. B.	3,050	10	03	129.8	138.5	41.5
M. J.	2,675	5	30	134.4	160.4	40.6
G. H.	2,730	5	07	110.3	123.3	34.7
R. D.	2,320	4	28	123.8	139.2	42.0
E. M.	3,380	4		123.7	151.6	44.3
H. B.	3,850	3		114.0	115.8	26.0
C. W.	3,160	2	43	116.3	156.0	54.1
T. L.	2,710	2	25	122.3	127.6	32.0
Average	3,060			122.6	138.7	38.6

* Four milligrams of 2-methyl-3-phytyl-1,4-naphthoquinone equal 2,000 units.

level, as recorded in Table VII, was elevated to 49.6. This not only represents a significant elevation, but is the greatest elevation obtained with these small doses.

Table VIII records five deliveries, the mothers having been given 2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate, intravenously, during labor. The mean infant plasma prothrombin level is shown to be elevated to 48.5 units. While it would seem futile to subject such a small series to statistical analysis, this procedure as recorded in Table IX demonstrates a very significant difference when

TABLE VII

*The plasma prothrombin levels of mother and child, expressed in dilution units, in twenty-seven full term deliveries following treatment of the mothers with one milligram of 2-methyl-1,4-naphthoquinone during labor**

NAME	INFANT WEIGHT	HOURS FROM ONSET OF TREATMENT TO DELIVERY		PROTHROMBIN DILUTION UNITS		
				Mother prior to treatment	Mother at delivery	Infant at delivery
		hours	*minutes*			
S. L.	3,190	22	01	161.2	188.2	46.7
S. A.	3,170	13	28	175.2	209.6	64.4
R. M.	2,815	13	12	115.4	129.8	20.3
V. C.	5,030	12	45	116.4	148.3	53.5
E. W.	4,200	12	41	186.4	216.7	13.9
A. H.	3,430	8	50	189.8		57.2
L. S.	3,250	7	50	101.7	121.2	32.5
A. R.	3,040	6	45	102.0	123.3	37.2
E. H.	3,300	4	44	174.0	207.7	60.4
R. B.	2,015	4	33	127.2	147.5	50.5
M. M.	3,600	4	23	95.7	108.4	33.5
E. J.	3,700	4	10	146.3	202.1	94.8
E. L.	3,210	4	10	186.0	251.4	69.4
B. H.	3,275	4	05	117.8	142.6	45.5
A. H.	3,000	3	40	143.9	162.3	53.9
C. J.	2,690	3	35	109.4	135.1	36.0
V. A.	2,730	3	30	139.3	166.7	43.2
M. B.	2,975	3	28	154.4	175.2	46.6
R. P.	2,180	2	22	120.2	145.5	40.3
H. M.	3,560	2	20	99.2	111.6	13.0
F. J.	2,730	2	19		207.9	79.9
M. W.	3,470	1	30	142.9	190.2	60.7
E. M.	1,900	1	05	108.4	133.9	40.2
A. C.	3,425	1	05	237.3	256.5	72.0
V. F.	2,460	1	01	214.2	259.2	49.3
N. K.	3,030	1	01	131.6	182.3	68.4
G. G.	2,200	0	18	117.3	151.3	57.6
Average				137.5	165.7	49.6

* One milligram of 2-methyl-1,4-naphthoquinone in corn oil prepared in gelatin capsules (one milligram equals 2,000 units).

contrasted with the control group. Such an analysis is excusable in view of the uniformity of the results obtained in each instance.

Thus, in 2,000 unit doses, three synthetic compounds given by mouth, and one given intravenously, have been shown to elevate

significantly the infant plasma prothrombin level when given to the mothers during labor, namely, 2-methyl-1,4-naphthoquinone, 2-methyl-1,4-naphthohydroquinone dipropionate, 2-methyl-3-phytyl-

TABLE VIII

*The plasma prothrombin levels of mother and child, expressed in dilution units, in five deliveries following treatment of the mothers with 0.5 cc. of 2-methyl-1,4-naphthohydroquinone-3 sodium sulfonate, intravenously during labor**

NAME	INFANT WEIGHT	HOURS FROM ONSET OF TREATMENT TO DELIVERY		PROTHROMBIN DILUTION UNITS		
				Mother prior to treatment	Mother at delivery	Infant at delivery
		hours	*minutes*			
H. L.....	3,580	17	35	141.5	159.5	49.3
M. W.....	2,600	6	45	141.1	160.4	47.9
A. B.....	3,210	5	30	105.6	143.4	48.1
M. M.....	3,830	4		112.1	146.3	45.9
G. S.....	2,755	1	12	104.1	136.7	51.5
Average....	3,195			120.9	149.2	48.5

* 0.5 cc. 2-methyl-1,4-naphthohydroquinone-3 sodium sulfonate is equivalent to one milligram of 2-methyl-1,4-naphthoquinone.

TABLE IX

Statistical analysis of infant plasma prothrombin levels

TABLE	NUMBER OF OBSERVATIONS	MEAN	S.D. OF OBSERVATIONS	S.D. OF MEAN	S.D. OF S.D.	S.D. OF DIFF. MEAN	DIFF. IN MEANS / S.D. DIFF MEAN
I	32	28.6	11.53	±2.0	±1.4		
II	12	39.9	25.35	±7.3	±5.1	7.59	1.4*
III	10	32.7	14.26	±4.5	±3.1	2.48	1.6
IV	12	35.2	8.6	±2.4	±1.7	2.93	2.2
V	17	39.2	5.75	±1.3	±0.9	2.46	4.3
VI	10	38.6	8.81	±2.7	±1.9	3.45	2.8
VII	27	49.6	19.23	±3.6	±2.5	4.20	5.0
VIII	5	48.5	1.83	±0.9	±0.6	2.19	9.0

* The means are considered to show a significant difference from the mean of the control series if the ratio of the difference of the means to the standard deviation of the difference of the means is 2.5 or greater.

1,4-naphthoquinone, and 2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate. In the same dose vitamin K concentrate was not significantly effective. Similarly, substances of demonstrated

potency, when administered in insoluble milk-sugar pills or intramuscularly, failed to produce significant results. The infant plasma prothrombin level was raised to the highest level by the administration of substances of the simplest chemical structure. This is doubly apparent when it is realized that in order to administer unit equivalent doses of these compounds, correspondingly greater amounts of the more complex substances had to be given. This bears out the contention of Ansbacher, Fernholz, and Dolliver (5) that 2-methyl-1,4-naphthoquinone possesses the greatest vitamin K activity of all the synthetic substances so far described. It suggests, moreover, at least on a theoretical basis, that the body metabolizes the more complex compounds to their simplest form before utilizing them in the elevation of the plasma prothrombin. That the substance must be readily available to the body for use is also well demonstrated.

SUMMARY

1. In small, unit-equivalent doses, 2-methyl-1,4-naphthoquinone, 2-methyl-1,4-naphthohydroquinone dipropionate, and 2-methyl-3-phytyl-1,4-naphthoquinone, dissolved in oil are capable of raising the plasma prothrombin of the newborn infant when given by mouth to the mothers during labor.

2. In similar doses intravenous 2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate is shown to possess significant vitamin K activity.

3. On the contrary, under identical conditions, 2-methyl-1,4-naphthoquinone in milk-sugar pills, and vitamin K concentrate, by mouth, and 2-methyl-1,4-naphthohydroquinone-3-sodium sulfonate, intramuscularly, could not be shown to be effective in raising the plasma prothrombin levels of the newborn infants.

Vitamin K active substances submitted by E. R. Squibb Company, and Abbott Laboratories, Inc.

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THE PATIENT AS A PERSON[1]

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Probably everyone has at least a vague idea of what is meant by the phrase, "The Patient as a Person." Nevertheless, it seems desirable at the outset to limit and define it in order that we may have, if possible, a clear understanding of what we are to consider. I shall discuss the patients that are admitted to the wards and out-patient departments of our general hospitals, that are given medical care by hospital staffs rather than by private practitioners. They are in a sense public patients, as contrasted to private patients who choose their own doctors, but this distinction has no bearing on their status as persons.

A person is a human being who is designated as a patient when under medical care, and it is in this situation that we are to consider him. We wish to discuss the patient as a total individual and in so doing depart from the traditional method of medical science which for purposes of study, simplification, and classification, divides man not only into systems and organs, but even down to individual cells. This division is necessary in order to gain a penetrating knowledge of the human body and of its many derangements of structure and of function that constitute disease.

The human body, however, is not the human being, but only a part of a person. The human being is a unity of body and mind, and is not even limited by the confines of the single, individual body. The human being may be defined as a member of the human race, and is not, except under very exceptional circumstances such as a Robinson Crusoe or a prisoner in solitary confinement, an isolated, single member. Much that goes to make up the characteristics of the human

[1] An address to the Social Service Committee of the United Hospital Fund of New York.

being or of a person is dependent upon almost constant and ever changing human relationships. Human beings, or persons, may therefore, be spoken of as social beings, each one having a definite place in society determined by fate, tradition, inherent qualities, and opportunities.

I wish to give particular emphasis to social relations, or to human relations if you prefer, and to show their significance in illness. The philosopher, John Dewey, suggests that the fine old saying, "a sound mind in a sound body," should be rephrased, "a sound human being in a sound human environment," and he urges that in the study of this matter, the physician has a position of unique opportunity and responsibility.

It seems appropriate to discuss the relation of human environment to illness, because it is a field of medicine especially cultivated by medical social service. I shall comment briefly on three aspects of the social or human relations of the patient as a person, and shall recount some of my own experiences in the study of this field. These three aspects are social disturbances as a cause of illness, social disturbances as a result of illness, and the problem of restoring the patient to his normal place in society, involved in that rather vague term, "convalescent care."

In order to illustrate social disturbances as a cause of illness, I wish to refer to a study of a series of fifty patients with digestive disturbances, admitted in rotation and studied without selection in the Gastrointestinal Clinic of the Johns Hopkins Hospital Dispensary. Patients with digestive disturbances, or with indigestion, to use the popular term, were we knew, frequently ill because of emotional disturbances, and were without organic disease, but we were not prepared to find as we did, that adverse social conditions were entirely or importantly related as a cause of illness in forty-four of the fifty patients. In thirty of them no evidence of organic disease could be discovered. A few of these cases serve to illustrate what is meant by adverse social conditions as a cause of illness in patients without demonstrable disease.

A 31 year old Polish housewife had been ill for two years. Her husband was an attractive Portuguese machinist who, besides caring for his wife and two children, supported his wife's mother and father.

The latter, a Polish trained cabinet-maker of 63, had had almost no work for six years. In spite of his situation he endeavored to dominate the house-hold, and friction between him and his son-in-law was a constant cause of severe emotional strain on the patient. Her attitude toward the home situation, and that of her husband toward "the old man," was readily adjusted when the cause of her illness was shown to them both. She learned to laugh at the situation rather than cry, and her symptoms soon disappeared. Nine months later she reported continued good health.

A 20 year old baker with severe and disabling symptoms, had a fear of tuberculosis because he had seen a young man, his next-door neighbor, die of the disease soon after his first child was born. The patient's symptoms began with his marriage and gradually increased as his wife neared the time of her first confinement. His fear was kept constantly before him by his mother, who told him that he would die of tuberculosis if he did not stop playing the saxophone.

Another patient, a skilled worker in a shoe factory, had digestive symptoms for years, which had finally brought him to idleness and public relief. The only discoverable cause for his illness was a constant loss of temper with a foreman who had been a source of irritation for a long time.

These three patients became quite well and returned to work after their illness was explained to them and after they had gained an insight into the true nature of their symptoms.

These cases are typical of thirty out of the fifty studied, and are good examples of adverse social conditions as a cause of illness. The patients were all carefully examined, first of all to determine their physical status, and were then given an opportunity to talk in a quiet office away from the activities of the dispensary, about the problems of their past and present lives. The idea was to combine the thorough study of the specialist with the attitude and approach of the family physician. It was surprising how frequently these patients revealed adverse social conditions which had caused emotional strain and gave the clew not only to the cause of their illness, but to the necessary steps for their successful treatment.

In nearly all the patients in this group, marked and sustained improvement or cessation of symptoms occurred, because a means was found by which their emotional strain could be lessened or abol-

ished, through a study of their social situations and environment. These results were, however, not always possible, because in a few instances no radical improvement in the situation could be brought about, and because the patients had already passed through such severe strain that their resistance to adversity had been, as it were, completely used up.

A striking example of this condition was seen in a 49 year old housewife, who said at the outset of the first interview that her life would make a good novel, and that she was sorry she was not a writer. She was the fifth child in a family of 21 or 22 children, and described her father as a gambling, horse-trading, drunkard, who beat his children and drove them away from home. At the age of seven she was badly burned and scarred when her father's house burned down. At twelve she ran away to escape the ill-treatment of her father and married a boy of twenty. When sixteen, a son was born and four years later her husband was killed. She then had to return to her father's house, but was forced to leave by his cruelty, two years later, with her six year old son. She made her way with a brother from the Middle West to Maryland. After supporting herself and her son for six years, she married a fisherman who lived on an island in the Chesapeake Bay. Seven years before coming to the hospital, her husband began to lose his vision, and two years later, when he had become almost completely blind, a storm had washed away their home and all their belongings. For several years the patient had been without news of her son, and she was apparently more disturbed by this loss and uncertainty than by her husband's blindness. She and her husband were doing the best they could, living in one of the poorest sections of Baltimore on a blindness pension of thirty dollars a month. It was no wonder that this patient had digestive and other symptoms, including attacks of unconsciousness which were apparently hysterical in nature, and it was no wonder that her health could not be much improved.

In the group of patients we are discussing, the social problems were studied by a doctor, but under usual conditions of hospital practice, when an adequate number of well-trained medical social workers are available, much the same information can be obtained by them. In order that they may carry out successfully the therapeutic work which should be their ultimate objective, there are two requisites, time to

make a thorough study, and close collaboration with doctors. Unfortunately, these two requisites are too often unattainable by medical social workers, and the foregoing cases are examples of what they may accomplish under favorable conditions which exist today in comparatively few hospitals. These cases serve, nevertheless, to illustrate social disturbances as a cause of illness.

The second aspect of the subject of the patient as a person consists of detrimental social disturbances as a result of illness. This social aspect of illness may be discussed quite briefly, starting with the familiar relationship of illness and poverty. A prolonged or chronic illness, incapacitating the bread-winner of a family or the mother of dependent children, has long been recognized as creating situations where the help of social agencies or public relief is urgent. But when we look closely at these situations and study the persons involved, we see more than mere poverty. We see emotional strain, discouragement, torn affection, and sometimes a relinquishment of the desire to live.

I have followed families in whom a member had developed tuberculosis, and have studied the effects of the disease on the family situation and on the individuals involved. Quite recently, there appeared in the dispensary, a neatly dressed, good spirited man, who had been treated for tuberculosis in a public hospital, which he had left against advice. Inquiry revealed that in spite of his need for further treatment, he could not be readmitted, because he was considered uncooperative. The man and his wife were interviewed by the social worker, and his home was visited not only by the public health nurse, but also by the medical student who was especially interested in him. This man had had a steady job as a gas-station attendant and was taken ill quite suddenly. He had been admitted to the hospital within a few weeks after his first symptoms had been noticed, and was a favorable case for pneumothorax treatment. His wife and three young children were promptly given public relief, and the situation seemed to have been cared for as adequately as possible.

After about four months in the hospital, the patient was given a few hours leave of absence to visit his family and failed to return to the hospital. When seen in the dispensary about a month later, his disease had progressed, but he resisted the proposal that he should have further sanitorium care. It was then learned that at the time

of his visit from the hospital he had found his wife ill in bed and all the children with bad colds. His wife expected to have another baby in about three months and had no relatives to care for her. The patient chose to stand by his wife, who was much disturbed emotionally, and his children, even at the risk of his own health, rather than to return to the hospital for further treatment. He did not fully realize how much his own condition was a menace to his children, but even so, his choice seemed justified and he was courageous rather than uncooperative. When all the facts were known, the patient was isolated at home under the supervision of a public health nurse, to remain there until the whole situation could be worked out satisfactorily for his wife and for him, which included proper care for the mother during her confinement and for the children during her convalescence. The patient was then ready to go through with the prescribed treatment, and only after these arrangements were made was it possible for him to carry out the sort of rest and relaxation that he needed.

I have recently seen several patients with tuberculosis whose disease ran an unexpectedly rapid and unfavorable course, because of the emotional strain resulting from the adverse social conditions in their families produced by the necessary sanitorium care, or other difficulties of their illness.

Chronic heart disease is another common cause of social disturbances which are detrimental to patients and very difficult for family groups among poorer people. Many examples of the effects of this disease, of epilepsy, and of other chronic illnesses could be cited from recent experience to illustrate this point, but it is not difficult for anyone familiar with illness to appreciate the necessity for a comprehensive study of the whole situation surrounding patients from underprivileged groups with prolonged or chronic illness. Only by a broad study of such patients as persons is it possible to make the most out of their damaged lives and to guide, encourage and console them and those around them in a brotherly and tolerant spirit and with professional insight. Here is another field of hospital care in which medical social service should play an indispensable part.

The third aspect of the patient as a person to be considered involves a period of illness called convalescence, a period between the cessation of disease or its acute manifestations and the final restoration of the

patient to his normal place in his usual social setting. This is today a relatively neglected period in hospital care, for which there are inadequate facilities throughout this country, and of which comparatively little exact knowledge exists. It is a field of medical care in which the study and treatment of the patient as a person is of the greatest importance, as it requires understanding of the mind as well as of the body and also of the social situation to which the patient is, if possible, to be restored. There are hazards of convalescence, just as there are hazards of disease, about which little is known or appreciated in present day hospital practice.

As an example of these hazards of convalescence, the case of a patient recently admitted to the Johns Hopkins Hospital Dispensary may be cited. He was a 34 year old industrial worker, who had had an attack of rheumatic fever at the age of 12, involving his heart and leaving behind an easily detectable heart murmur. This murmur had been heard by a doctor when the patient was 19 years old and again a few years ago when he was given a routine examination for employment. During these years, however, he had withstood without symptoms strenuous physical work in the steel industry, as a furniture mover, and as a soldier in the National Guard. It seemed clear that in spite of his heart murmur, his heart was functionally sound, and had caused no physical disability. Over two years ago the patient had been severely injured in an automobile accident, and had been confined in a hospital for 13 weeks. Since his discharge he had been a semi-invalid, unable to work, and living with his wife and two small children in a dark, cheerless, low-rent flat, supported by public relief. His symptoms were not those of heart disease, but were characteristic of anxiety and emotional strain. This patient revealed in an interview that his illness was apparently the result of what happened to him after his injuries, including a fractured pelvis, had healed. He was "nervously" shocked by his accident and injuries and by the warnings that his heart was damaged, rendering him unfit to enter again his accustomed trade as a steel worker. He had seldom gone out of his home for two years. He therefore presented the problems of evaluating anew the amount of work he could safely undertake, of giving him an understanding of the significance of his symptoms, with appropriate measures for the relief of his anxiety and emotional tension, and of helping him to find suitable employment that would

not continue to be a source of anxiety to him. After seeing his home, his wife and children, and after a study of his mental attitude, his education, which was rather superior, and his other capabilities, there was reason to believe that he could be returned to a state of self-support, good health and a happy life.

The study and treatment of such patients during their periods of social adjustment require close collaboration of the medical social worker and the doctor, and such team-work may be a large factor in the ultimate success of medical care.

Seriously ill persons frequently have to be adjusted mentally to the requirements of medical care. They are relieved as far as possible of responsibilities, they are admitted to hospitals where they have no decisions to make, they are given attention to which they are unaccustomed when well, and they are expected to think, to act, and to conform to certain patterns of behavior very different from their habitual manner of living. During convalescence, much of this adjustment has to be corrected, and patients have to learn to resume responsibilities, to alter again their behavior, and to stand upon their own feet once more. Some patients are eager to do this, but to others, illness or convalescence has such alluring compensations that it is convenient or satisfying to prolong it as far as possible. Each patient needs to be treated, therefore, as a unique individual, and the unity of the human being must be constantly borne in mind. There is much to be learned about convalescence and its management, but the study of the patient as a person, in which the social worker and doctor must constantly cooperate, is evidently the most dependable approach to this problem.

In closing, it is hoped that some of the problems of the patient as a person have been presented in a way to encourage those who are interested in hospital care to see its bearing on the treatment of the patient. The study of the social problems of hospital patients is essential in order to determine factors causing illness, to evaluate and relieve the effects of illness, and to complete restoration of health. In this field, medical social service must take an important and cooperative part, but the ultimate responsibility and guiding hand must be that of the medical profession. It is here that an awakening interest in the patient as a person gives us much reason for encouragement.

THE NATIVE HORMONES OF THE POSTERIOR PITUITARY GLAND: THE PRESSOR AND OXYTOCIC PRINCIPLES[1]

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Conclusive evidence has been presented by Dudley (1), by Kamm, Aldrich, Grote, Rowe and Bugbee (2), and by Stehle and Fraser (3) that extracts of the posterior pituitary gland may be prepared and fractionated in such a manner as to yield two separate products, a pressor and an oxytocic principle. It has been implied that these separated principles exist as such in the pituitary gland, and such fractionation has been adduced as evidence in support of the multiple hormone theory. Abel, Rouiller and Geiling (4), and Abel (5) contended that the physiologically active constituent of the posterior pituitary gland is a large molecule with multiple activities, and that this unitary hormone can be readily hydrolyzed into physiologically active cleavage products.

An examination of the molecular dimensions of the various pituitary principles would aid materially in detecting the presence of such a large hormone molecule and should establish the point whether or not the substances obtained after certain chemical manipulations are cleavage products resulting from dissociation of the native hormone.

Sedimentation properties of the pituitary principles have been examined in the Beams (6) vacuum type air-driven centrifuge.[2] The qualitative observations available at present offer definite answers to certain of these problems.

[1] This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

[2] Built by Messrs. Linke and Sommer, Charlottesville, Va.

PROCEDURE

Studies were carried out upon the three commercial preparations Pitressin, Pitocin and Pituitrin (Parke, Davis and Company) and upon an extract prepared in the laboratory. In order to avoid exposure of the hormone constituents to injury during chemical manipulations the posterior pituitary extract was prepared as a press-juice following a procedure similar to that used by Irving and du Vigneaud (7). Six pituitary glands of cattle were removed a few minutes after death and were frozen in a tube surrounded by dry-ice.[3] The glands were thawed for dissection and then the posterior lobes were alternately frozen and thawed 5 or 6 times with the intention of rupturing the cell walls. The intact glands were inserted into a chilled pressure chamber, and the tissue juice was expressed in a Büchner hydraulic press at 30,000 lb. per square inch. The press-juice was taken up in 16 cc. of ice-cold 0.9 per cent solution of sodium chloride. After a preliminary centrifugation at 2000 r.p.m. in chilled tubes, the opalescent solution was centrifuged at 60,000 r.p.m. a few minutes to yield a crystal clear supernatant fluid. This clear solution was transferred to fresh tubes for the final centrifugation at 61,000 r.p.m. for $5\frac{3}{4}$ hours. At the conclusion of the run 5 equal fractions, about 0.9 cc. each, were carefully drawn off from each tube with a fine pipette whose tip was bent upward. Each of the fractions was stabilized by adding an equal volume of 0.5 per cent acetic acid and bringing the material quickly to a boil. Samples were numbered consecutively from 1, the top fraction, through 5, the bottom fraction. Pituitrin, Pitocin and Pitressin were each diluted before centrifuging by the addition of 2 parts of 0.9 per cent sodium chloride solution to one part of the original ampule (1 cc. = 10 or 20 International Units).

The results reported here were obtained principally upon materials centrifuged simultaneously in the same run for a period of $5\frac{3}{4}$ hours at 61,000 r.p.m. in a 6 inch angle rotor. The field was approximately 250,000 times gravity at the lower part of the inclined test-tubes, each of which held 4.5 cc. of fluid. The rotor was chilled in ice water just

[3] I wish to thank the Schluderberg, Wm.-T. J. Kurdle Co., Baltimore, Md. (Mr. J. C. Kistler, Director of Laboratories), for their generous supply of fresh gland material.

before operation but its temperature rose during centrifugation to about 15°C.

Concordant results on the press-juice were obtained in 3 centrifugations, one for 2 hours at 60,000 r.p.m., one for 4 hours at 60,000 r.p.m., one for $5\frac{3}{4}$ hours at 61,000 r.p.m. Pituitrin was included in the last two runs, Pitressin, Pitocin, and acid treated extract were centrifuged once in the final $5\frac{3}{4}$ hour run.

Pressor activity was standardized on the basis of the blood pressure response in cats anesthetized with sodium phenobarbital. Oxytocic activity was assayed on strips of virgin guinea pig uterus.

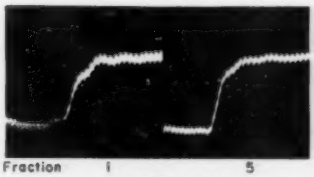
RESULTS

Pituitrin is a solution of posterior pituitary that has been purified to remove inert protein. It retains the combined pressor and oxytocic activity of the pituitary gland. The samples were therefore examined for both activities. Record 1 shows that the pressor action of fraction 1 is only slightly less than that of fraction 5. In Record 4 it is seen that the top fraction 1 is only slightly weaker in oxytocic activity than the lower fractions. The apparent differences between successive fractions are somewhat exaggerated in Record 4 because the uterine strip used for this test became hypersensitive toward the end of the experiment. The topmost sample was estimated to contain about 25 per cent less pressor and oxytocic substance than the bottom fraction. The two activities represented in Pituitrin sediment at a slow rate and can therefore be attributed to small molecules which might well be of the order of 600 in molecular weight, the value ascribed to them by Kamm (8).

The pressor activity of Pitressin also sedimented slowly. In Record 2 the top fraction 1 shows very little reduction in pressor action compared to fraction 5. This represents approximately a 25 per cent difference in concentration of active material.

The oxytocic activity of Pitocin exhibited weak sedimentation of about the same degree as that observed in Pituitrin and Pitressin. An assay, Record 5, of the various layers in the tube of Pitocin, gave fraction 1 a value of 70 per cent in concentration of oxytocic substance compared to the bottom fraction 5.

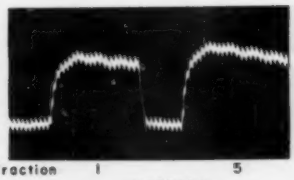
The sedimentation rates of the two activities present in the un-



Fraction 1 5

PITUITRIN

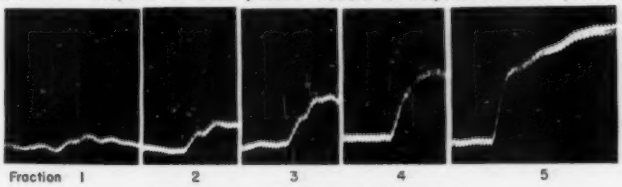
Record 1 Response of blood pressure



Fraction 1 5

PITRESSIN

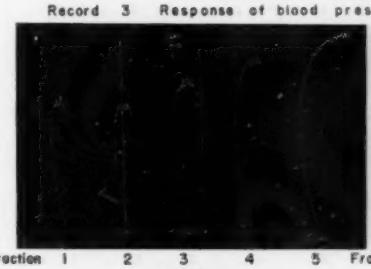
Record 2 Response of blood pressure



Fraction 1 2 3 4 5

POSTERIOR PITUITARY PRESS-JUICE

Record 3 Response of blood pressure



Fraction 1 2 3 4 5

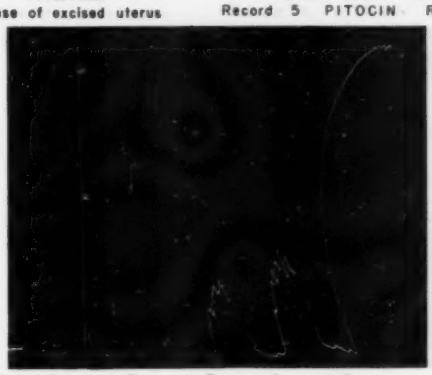
PITUITRIN

Record 4 Response of excised uterus

Fraction 1 5 1 5

.6cc .3cc .5cc .4 cc

Record 5 PITOCIN Response of uterus



Fraction 1 2 3 4 5

POSTERIOR PITUITARY PRESS-JUICE

Record 6 Response of excised uterus

FIG. 1

treated press-juice stood in striking contrast to the rates observed in the case of Pituitrin, Pitressin and Pitocin. Record 3 shows the relative pressor activities of the samples, while Record 6 illustrates the parallel series of responses of the guinea pig uterus to the same samples. In each case fraction 1 and fraction 2 show barely perceptible activity, while the bottom fraction 5 exhibits a high degree of concentration of active material. An assay showed that fraction 1 contained about 2 per cent of uterine stimulant material as compared to fraction 5. This may be compared to the results in the case of Pituitrin, Pitressin and Pitocin where fraction 1 had about 75 per cent of active substance compared to the bottom fraction.

In order to ascertain whether the two activities sedimented at the same rate in untreated press-juice, the lower 3 fractions—3, 4, and 5—were submitted to quantitative assay. The concentrations of the oxytocic constituent stood in the proportions $C_3:C_4:C_5 = 1:2:12$. An identical ratio for the same three fractions was found in the case of the pressor constituent. In another 4 hour run at 60,000 r.p.m. 3 fractions were removed. The concentration ratio between the top and bottom fraction in oxytocic constituent was $C_1:C_3 = 1:6$. The same ratio was obtained in the blood pressure assay. Within the limits of accuracy of the measurement (estimated at ± 15 per cent) the pressor and the oxytocic constituents of the untreated press-juice sediment at about the same rate. The two activities therefore reside either in a single large hormone molecule, the unitary principle of Abel, or in two separate large hormone molecules which are nearly alike in their rates of sedimentation.

A provisional estimate of the molecular size of native posterior pituitary hormone might be hazarded. In the 4 hour run at 60,000 a solution of twice recrystallized egg albumin showed a concentration

FIG. 1. Record 1, 2 and 3. Carotid blood pressure response in cats anesthetized with sodium phenobarbital. Pituitrin: 0.066 cc. of original ampule (1 cc. = 10 i.u.) at each injection. Pitressin: 0.033 cc. of original ampule (1 cc. = 20 i.u.) at each injection. Posterior pituitary press-juice: 0.01 cc. of original extract at each injection. Volume made up to 0.2 cc. for each injection.

Record 4, 5 and 6. Virgin guinea pig uterus suspended in 30 cc. Locke-Ringer's solution. Pituitrin: 0.6 cc. of 1 to 150 dilution of original ampule (1 cc. = 10 i.u.) at each addition. Pitocin: indicated volume of 1 to 150 dilution of original ampule (1 cc. = 10 i.u.). Posterior pituitary press-juice: 0.3 cc. of 1 to 500 dilution of original extract.

distribution roughly placed at $C_1:C_2:C_3 = 0:2:10$. The oxytocic and pressor constituents were distributed in the ratio of $C_1:C_3 = 1:6$. On this basis one would attribute to the native hormone a sedimentation constant of the order of $\frac{1}{2}$ or $\frac{1}{3}$ that of egg albumin.

The step in the usual chemical extraction procedure which leads to cleavage of the native hormone is indicated in an experiment on the acid treatment of the pituitary extract. To 5 cc. of the press-juice solution was added an equal volume of 0.5 per cent acetic acid. The solution was brought quickly to a boil, then filtered, chilled and centrifuged $5\frac{3}{4}$ hours at 61,000 r.p.m. The blood pressure record showed the presence of both slowly and rapidly sedimenting constituents indicating that a portion of the native hormone had undergone splitting into small but active products.

SUMMARY

The pressor and oxytocic principles exist in untreated press-juice of the posterior pituitary lobe in the form either of a single large molecule or of two separate large molecules which are very similar in their sedimentation properties. Because of the mild extraction procedure used this large molecular species is regarded as native pituitary hormone. A provisional estimate places the sedimentation constant of the native hormone at a value about $\frac{1}{2}$ or $\frac{1}{3}$ that of egg albumin.

Pituitrin, Pitocin, and Pitressin contain the active principles in the form of physiologically active cleavage products which are much smaller in molecular weight as compared to the native hormone. By momentary boiling in 0.25 per cent acetic acid the native hormone can be cleaved in part to yield physiologically active split products.

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PROCEEDINGS OF A MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

Held in the Hurd Memorial Hall, April 8, 1940

The President of the Society, Dr. G. Canby Robinson, presided. Abstracts of the papers and discussions follow:

Experimental Studies of Sulfathiazole as a Urinary Antiseptic. By Justina H. Hill. (Brady Urological Institute, The Johns Hopkins Hospital.)[1]

In a case of Staphylococcus aureus septicemia the free sulfathiazole in the urine reached a level of 258.5 mg. per cent. At pH 6.20 this urine not only sterilized itself, but could kill in 24 hours as high as from 1 billion to 4 billion bacteria per cc., the test organisms being Staph. aureus, alpha Streptococcus faecalis, Aerobacter and Proteus. If the equivalent amount of drug was added to normal urine, although marked reduction of these high numbers of organisms was obtained, the results were less striking than in the patient's urine. This finding has been repeatedly confirmed.

The increased action *in vitro* cannot be attributed to the intermediate forms tested, that is, acetyl-sulfathiazole, 2-amino-sulfathiazole, acetyl-2-amino thiazole and 2-amino 4-phenyl thiazole. Similarly, 2-amino 4-methyl thiazole and acetyl-methyl thiazole had little or no effect, while methylsulfathiazole was only slightly less bactericidal than sulfathiazole.

It was found that while the original observations of Long and Bliss, that peptone interfered with the action of sulfanilamide, were at once confirmed by our experiments with Escherichia and Aerobacter, no such interference was observed in the case of sulfathiazole at the 100 mg. per cent level. With para-amino-benzoic acid which has recently been reported by Wood and Fildes to block the antibacterial action of sulfanilamide, this action was confirmed with 0.1 per cent para-amino-benzoic acid added to 100 mg. per cent of sulfanilamide in urine pH 6.40, tested against Escherichia (2, 415 or 13,125 organisms per cc). Complete sterilization was obtained under the same conditions with sulfathiazole. Further experiments indicate, however, that with other organisms, some interference may be obtained with para-amino-benzoic acid in the case of sulfathiazole.

Comparison of 0.01 molar equivalents of glycine and of hippuric acid, as suggested by Dr. E. C. White, although inactive themselves, showed no interference

[1] The author wishes to acknowledge the assistance of Dr. George Harrop and of Dr. W. A. Lott, of the Squibb Institute for Medical Research, who kindly provided the intermediate compounds used in these studies.

with the action of 100 mg. per cent of sulfathiazole in urine against Escherichia. 0.01 molar para-amino-benzoic acid itself was actively germicidal for this organism.

The action of sulfathiazole upon Aerobacter, Proteus, Pseudomonas pyocyanea and different types of streptococci was shown to be powerful, and the influence of the number of organisms was emphasized. Group D beta streptococci were not killed but definite action was obtained against alpha and gamma strains of Streptococcus faecalis, the streptococcus most frequently observed in urinary tract infection. But it seems probable that the faecalis types of streptococci will be more resistant to sulfathiazole than other types of organisms.

Upon the findings reported here of the direct germicidal action of sulfathiazole, the recommendation is made that it be given adequate clinical trial in cases of genito-urinary infection, provided such other factors as toxicity are also favorable.

DISCUSSION

Dr. J. A. C. Colston said that Miss Hill had asked him to say a few words about the clinical experience with sulfathiazole in the Brady Urological Institute. He said that this experience had been limited, for the drug had been used clinically for only six to eight weeks and that any conclusions from such a brief experience must be guarded.

The drug had been used in eleven cases of gonococcal anterior urethritis with excellent results. Only one case of gonococcal anterior urethritis had failed to respond satisfactorily and in that instance the blood level had failed to rise above 3 mgm. per cent. No hematuria and no other complications had been encountered in this group of patients.[2] Sulfathiazole had also been used in fifteen cases of urinary tract infection. In one case it had to be discontinued because of nausea and vomiting. In one case in which a staphylococcal pyelitis was complicated by staphylococcal septicemia, the illness progressed and the patient died, in spite of large doses of sulfathiazole (9 gms. per day). In the other urinary tract infections, mostly mixed infections, the results have been satisfactory to date. The only complication encountered was fever and conjunctivitis which occurred in three cases.

Our present feeling is that sulfathiazole will probably prove to be more valuable than sulfanilamide in gonococcal infections because it seems to be far better tolerated in ambulatory patients than is sulfanilamide. Our experience with the drug in coccal infections of the urinary tract has not as yet been sufficient to to draw any definite conclusions but we all feel that sulfathiazole is of definite

[2] Since this discussion, Dr. Ormond Culp, under whose care the majority of these cases have been, has reported that in three patients, under treatment in the Out-Patient Department, it was necessary to stop sulfathiazole, on account of leukopenia in one instance, and fall in hemoglobin in two other cases. Following withdrawal of the drug, the blood picture returned to its normal status in a few days and no serious consequences resulted.

value and may be the most effective therapeutic agent that we have available for combating this type of infection.

Dr. Perrin H. Long reviewed his experience in the treatment of 147 patients with sulfathiazole. He said that toxic complications had occurred in 21.8 per cent. The complications were the following: 5 hematuria, 2 hematuria with anuria, 10 fever and rash, 7 fever, 1 leukopenia, 7 conjunctivitis. The percentage of complications has been much higher than that encountered in the first 150 patients treated with sulfanilamide and sulfapyridine.

He said that Dr. Haviland had been experimenting with the relative solubility of acetyl sulfathiazole and acetyl sulfanilamide and had found that the former was three times less soluble than the latter. Acetyl sulfathiazole therefore tends to precipitate out more readily in fluid media, such as urine.

Dr. Long related briefly the experience which he and Dr. Bliss had recently had in testing the effect of sulfanilamide upon the colon bacillus when the latter was grown in completely synthetic media, consisting of a salt base to which had been added glucose and various amino acids. He said that they had been able to determine that certain amino acids interfere with the action of sulfanilamide. In the presence of certain combinations of amino acids sulfanilamide appears to act as a growth stimulant, rather than the amino acid acting in a manner to inhibit the bacteriostatic action of sulfanilamide.

The Nature of the Thromboplastic Substance of the Tissues. By W. H. Howell. (The Johns Hopkins University.)

The tissue used for extraction was the lungs. Hemophilic blood, with an average clotting time of 70 to 80 minutes, was employed to determine the thromboplastic activity of the extracts. Pig's lungs, minced and thoroughly washed with acidified water, were extracted with distilled water (pH 6.8). The extract was filtered through a Buchner funnel and precipitated by the addition of 1 per cent acetic acid to the point of flocculation. The precipitate, collected by centrifuging, was dehydrated with acetone and extracted first with benzene and then with petroleum ether. Neither of these extracts contained significant amounts of the thromboplastic substance. Extraction was then made with chloroform. This extract contained the active substance. If sufficient chloroform is used all of it may be removed. After filtration the chloroform was removed by evaporation in a current of air at room temperature, the residue was extracted with acetone, recovered by centrifuging and dissolved in faintly alkaline water. The opalescent solution has strong thromboplastic activity. This solution was precipitated by adding 1 per cent HCl to the isoelectric point, and the precipitate, after centrifuging, was spread in thin layers upon glass and dried before a fan. A waxy substance was obtained which is insoluble in petroleum ether, but dissolves quickly and completely in chloroform. Preliminary analyses gave a nitrogen-phosphorus ratio of 2 to 1, indicating a diaminophosphatid. The dry material and its solutions gradually lose their potency over a number of days. The dry material on extraction with absolute ethyl alcohol falls into two parts, one soluble in the

alcohol, and one, about one-fourth of the original material, insoluble. The alcohol soluble portion, after evaporating off the alcohol, gives a residue insoluble in petroleum ether but easily soluble in chloroform. It has little or no thromboplastic action. The alcohol insoluble portion is insoluble in lipoid solvents, but dissolves readily in feebly alkaline water to a clear slightly colored solution which has marked thromboplastic activity. This material gives a strong reaction for phosphorus and a positive biuret test, indicating the presence of a phosphorus containing protein. Work is being continued upon the nature of this substance and of the phospholipin with which it is associated.

Renal Function in Hypertensive Dogs. By LOUIS K. ALPERT and CAROLINE B. THOMAS. (Department of Medicine, The Johns Hopkins Medical School.)

When hypertension is produced in dogs by permanent partial occlusion of the renal arteries, there is a temporary reduction of renal blood flow. Using the phenol red clearance method, Warthin, in this laboratory, demonstrated that after about a month, the renal blood flow returns to its normal level. We have attempted to determine whether the renal blood flow in chronically hypertensive dogs can be made to vary over as wide a range as in normal ones. The change in urea clearance as influenced by different diets was observed. It has been shown by previous workers that on a high protein diet normal dogs have a much higher urea clearance than on a low protein one. This change in urea clearance is paralleled by variation in renal blood flow. This fact was utilized by us in the study of three dogs which had had hypertension (Goldblatt type) for two to three years. In each of these dogs a marked rise in urea clearance occurred on increasing the protein intake. This may be interpreted as indicating that under these conditions the total renal blood flow in chronically hypertensive dogs is not different from the normal.

DISCUSSION

Dr. James Bordley, III said that these experiments seemed to point to a very interesting field for future investigation. It is surprising to have information which appears to indicate that the renal blood flow and the elasticity of kidney function may be normal in dogs with "renal hypertension." Dr. Alpert made no conjectures as to why the hypertension persisted in these animals. Two possible explanations come to mind: (1) Although the total blood flow may be normal, the blood flow to some particular elements of the kidney—the glomeruli or the tubules, for example—may be relatively reduced; (2) Some recent experiments of Kohlstaedt and Page on the elaboration of pressor substances by the kidney have directed attention to the possible importance of the pulse pressure in the kidney; the latter may be reduced, while the total blood flow is normal.

Dr. Bordley said that he wished to say a few more words about Dr. Warthin's experiments which had been mentioned by Dr. Alpert. These experiments were carried out chiefly on five dogs. The method used for measuring the renal blood flow gave figures which were by no means exact and a reduction in renal blood flow of 10 per cent or even 15 per cent might have escaped detection. Unfor-

tunately, Dr. Warthin had to abandon this line of work when he left here last September and not enough experiments were completed to substantiate his conclusions beyond argument. However, it was interesting to find that his conclusions appeared to be supported by the work of Drs. Alpert and Thomas in which the approach has been from an entirely different angle.

Dr. Caroline B. Thomas pointed out that the three dogs used in their experiments had the "benign" form of renal hypertension. As yet they had made no observations concerning changes in urea clearance following variations in diet on dogs with the malignant type of hypertension described by Goldblatt. It seemed possible that the findings in the malignant form of hypertension would be quite different.

Observations on Hypertension among the Chinese People. By F. R. DIEUAIDE. (Massachusetts General Hospital.)

Various types of elevation of blood pressure were found in 1.4 per cent of about 50,000 Chinese and in 2.8 per cent of about 7,800 non-Chinese (overwhelmingly European) in-patients of the Peiping Union Medical College Hospital from 1921 to 1937. Among the Chinese, 213 well-studied cases of uncomplicated "essential hypertension" were selected. The corrected sex distribution was male 40.7 per cent, female 59.3 per cent. The age distribution showed the bulk of the cases to occur between 40 and 60, with a lower proportion in the seventh decade (corresponding to that in the ward population), than obtains in this country. The incidence did not appear to increase at an earlier age in women than in men. The levels of pressure resembled those seen in this country, but the highest values were less often attained. The symptoms listed, the associated disturbances, the results of examination, and the course, were essentially the same as those known for Europeans, except that frank clinical coronary occlusion was seen only once. Of the patients followed for 5 or more years, 15 never had any complaint. Familial incidence (9 per cent), association with obesity (40 per cent), with diabetes mellitus (17 per cent), and with the menopause (49 per cent of the women), were striking. No special social distribution was noted. Severe renal failure was perhaps less common and cerebral accidents were perhaps more frequent than one would expect. Seven patients died during the observations.

DISCUSSION

Dr. Nicholson J. Eastman said that when he was in China his experience with obstetrical patients had led him to believe that acute elevations in blood pressure occurred there with as great frequency as in this country. Eclampsia was seen frequently and eclampsia of a rather severe degree.

At the end of the meeting the following officers were elected for the ensuing year:

Dr. Warfield M. Firor—President
Dr. Edward S. Stafford—Secretary-Treasurer

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Synopsis of Digestive Diseases. By JOHN L. KANTOR. 302 pp. Illus. $3.50. *C. V. Mosby, Co., St. Louis, 1937.*

This pocket-sized book attempts "to present simply, clearly and concisely the essential facts . . ."; to "preserve the proper balance between stress and subordination, inclusion and omission; and to emphasize the ways into which gastroenterology fits into the larger field of internal medicine."

This huge task is accomplished as well as it can be done within the range of a compendium. While Kantor has critically utilized the current literature, his own viewpoints are primarily represented. These are to be respected in view of many years of teaching, clinical investigation and practice. However, biostatisticians may question some of his statistical methods and the resulting interpretations. There are brief, interesting chapters on the organic and functional constitutional inferiorities, gastro-intestinal allergy, digestive symptoms in extradigestive diseases and on intestinal parasitology.

As with all syllabi, the necessity for marked condensation leads to occasional unavoidable omissions and inadequate correlations, dogmaticism and to too many generalizations. There is danger in its use as a source of knowledge rather than as a guide. But this little book, like other adequate syllabi, should prove of distinct service and value if properly used: as an overview of some phase preliminary to study, to review, to give order, organization and perspective to the field, and as an aid to the teacher in knowing when the material has been completely covered. The value of Kantor's good synopsis would be enhanced were he to append references to several key articles at the end of each chapter encouraging necessary further study; none are now present.

M. P.

Die Ernährung der olymphischen Kämpfer in Vergangenheit und Gegenwart. By ADOLF BICKEL. 36 pp. Illus. Paper bound, R.M. 1.00. *Deutsche Verlagsgesellschaft m.b.H., Berlin, 1938.*

One is surprised to learn that the Olympic athletes nourished themselves instinctively in the same manner in the Olympic Games of 1936 as in the Olympic Games of antiquity. The food intake consisted chiefly of lean meat, to which were added 1–2.5 L. milk. The last food intake was 3 hours before the match and consisted of 3 beefsteaks! In an attempt to explain this high animal protein intake, Bickel emphasizes that during hard muscular work protein metabolism is

probably more important than is generally believed, and concerns muscular hypertrophy as well as muscular contraction.

Bickel reminds that during food intake rich in animal proteins the urinary ratios C/N and VO/N diminished, which indicates an increase in the oxidations of intermediary metabolic substances. During muscular work there was a tendency to decreased oxidation of the intermediary metabolic substances. This may be partly corrected by a high intake of animal protein. Bickel has shown that during this regime liver glycogen is at a low level, which possibly has some importance in muscular work.

D. K.

Ueber die Beziehungen der Qualität des Nahrungseiweisses zum Ablauf des Betriebsstoffwechsels. By ADOLF BICKEL. 100 pp. Paper bound, 10 Swiss francs. *Benno Schwabe & Co., Basel, 1938.*

Bickel makes a distinction between "Betriebsmetabolism" and "Baumetabolism." Nitrogen balances, oxygen consumption and weight variations give rough indications concerning the "Baumetabolism." It is a well known fact that in this respect the quality of proteins has as great significance as the quantity of protein intake, the organism being unable to synthesize some amino acids.

Measurements of respiratory exchange give information concerning the "Betriebsmetabolism," revealing the specific dynamic action of proteins. The urinary ratios C/N and Resting O/N give more important indications concerning the oxidations in the intermediary metabolism. In experiments on rats Bickel studies these urinary ratios in successive periods with various food intakes.

When the food intake was kept constant in quantity and quality the mean values of the ratios were quite constant during successive periods. Administration of protein (e.g. casein) irradiated with rays of mesothorium, instead of normal proteins, resulted in a decrease of the ratios. Administration of amino acids led to an increase in the ratios. Animal proteins resulted, in general, in lower ratios than vegetable proteins.

If the quality of the entire protein intake was suddenly changed, the ratios reached their new values within 24 hours. If there was only a partial change in the quality of protein large fluctuations of the ratios occurred and the new level was reached after 2–3 weeks.

Bickel tried to see if the quality of the protein intake had any influence on carbohydrate metabolism, the total nitrogen intake remaining constant. In rats vegetable protein intake was accompanied by high liver glycogen whereas animal protein intake was associated with lower values.

In conclusion Bickel stresses the importance which must be attached to the quality of the protein intake. By modifying the quality of protein it is possible to increase or decrease oxidation and increase or decrease liver glycogen.

D. K.

Introduction to Physiological and Pathological Chemistry. (*With Special Reference to the Needs of Schools of Nursing; with Laboratory Experiments.*) By L. EARLE ARNOW, Ph.D. 555 pp. Illus. $3.50. (*The C. V. Mosby Company, St. Louis, 1939.*)

To meet the needs of beginners, this text is divided into three parts: I, Introduction to Chemical Science, 130 pages; II, Physiological and Pathological Chemistry, 257 pages; and III Introduction to Laboratory Chemistry, 98 pages.

The author's announced purpose is to express the principles and concepts simply enough for an average student to understand them without additional explanation. This is conceivable; but considering the necessarily brief and occasionally superficial treatment of the introductory chapters to the whole of chemical science, one wonders if the thoughtful student will be content without further explanations, and if the "understanding" of the average beginner really means very much.

However, the sections dealing with physiological and pathological chemistry are generally sound and presented interestingly. There are frequent diagrams that are clear and instructive. (Some of the photographs are not well reproduced). The series of questions accompanying each chapter should help to better comprehension of the subject matter. The listing and discussion of all the alleged vitamins does not seem appropriate for an elementary text book such as this.

B. C.

Practice of Allergy. By WARREN T. VAUGHAN. 1082 pp. Illus. $11.50. *C. V. Mosby Company, St. Louis, 1939.*

The author has previously published two editions on allergy and applied immunology, intended for both physician and patient. The increasing amount of clinical and laboratory data accumulated in this field, during the past decade, prevents continuation of this plan and the present large volume of 1100 pages is therefore written for the physician only. The amount of information presented on most phases of allergy is adequate but on several phases it is inadequate. Beginning with an historical review of the relationships between allergy and anaphylaxis there follow the principals of diagnosis, physiology of allergy, disporportionately long sections on food and pollen allergy, and finally very brief consideration of specific types of clinical allergy. Urticaria, angioneurotic oedema, various types of eczema and perennial allergic rhinitis in which successful diagnosis and treatment are more difficult than in seasonal hay-fever, receive less attention than they deserve.

Portraits of many early prominent investigators in Immunology and Allergy are of interest. The many case analyses and the numerous good photographs, the latter taken by the author with a Leica camera, lend a personal touch not usually encountered in text books. The individual theories, experiences and opinions of the author are freely given but many of the latter touch on controversial and unsolved problems in allergy, and the reviewer finds himself frequently

in disagreement with the author. In discussing treatment of pollen hay-fever the statement "that the preseasonal dose must be large to be successful" and that "preseasonal treatment should not be attempted unless the patient reports within six weeks before the onset of the season," are assertions which would not be supported by the general experience of many allergists. Despite the occasional didactic trend of the author the book contains a vast amount of detailed and important information which is supplemented by a good bibliography and a complete index. It is recommended not only to those interested in allergy but also to those engaged in general medicine.

W. L. W.

Cancer—Its Diagnosis and Treatment. By MAX CUTLER and FRANZ BUSCHKE. Assisted by SIMEON T. PANTRIL. 757 pp. Illus. $10.00. (*W. B. Saunders Company, Philadelphia, 1938.*)

This volume is a very important one, for it deals with one of the foremost problems before the medical profession today, and it comes from the pens of two men who have had a vast experience with cancer, particularly in the field of irradiation therapy. The text is well written and well illustrated. Many of the illustrations are taken from the Hines Veterans Facility, Hines, Illinois, where the senior author is consultant to the tumor clinic and director of cancer research.

The world literature is well reviewed and there is an excellent bibliography.

The first chapter deals with irradiation therapy. Important in this chapter is a discussion of the radiosensitivity of tumor cells and the response of vasculoconnective tissue to irradiation. Following this there is a general outline of all the reactions one meets in irradiation therapy and the hazards of such therapy. There are chapters on biopsy and on the spread of cancer. Subsequent chapters deal with diagnosis and treatment of cancer and malignant tumors as found in all of the different organs of the body.

This is a good text for anyone entering the field of oncology and a good reference book for the general practitioner, as well as the surgeon, for it gives the choice of the method of treatment based on clinical results.

Unfortunately, the authors do not devote enough space to the physics of irradiation therapy, which is of paramount importance in cancer therapy today. Furthermore, the lack of detailed discussion of radium and roentgen-ray dosage is disappointing. The inclusion of such information would have made the book of greater value for consultation by all interested in the cancer problem.

G. E. W.

Psychopathia Sexualis. By RICHARD VON KRAFFT-EBING, M.D. Only authorized English adaptation of the last German edition revised by Krafft-Ebing. 626 pp., $3.00. *Pioneer Publications, New York, 1939.*

This is the latest edition of the well known and, in a way, history-making text of Krafft-Ebing, and is presented as the "only authorized English adaptation of the last German edition revised by Krafft-Ebing."

This book needs no introduction to the medical fraternity. It was an early landmark in the development of scientific interest in the sexual life. The material, viewed from this distance, appears to be little more than a catalogue of the varieties of sexual performance and especially of the sexual abnormalities. The explanations and some of the facts, as might be expected, do not serve any longer as explanations, but it may be added that present-day explanations of the same sorts of facts in the current lingo will also, in the long run, probably prove to be equally illusory.

It is a curious thing that Krafft-Ebing who was an all 'round important figure of his day should be remembered by posterity largely by this one work. He must have had considerable courage to amass and publish this material, and its significance in opening up a desire for fact finding in this field will always remain.

W. M.

Sketches in Psychosomatic Medicine. By SMITH ELY JELLIFFE. 155 pp. $3.00. *Nervous and Mental Disease Monographs, New York, 1939.*

These Sketches in Psychosomatic Medicine are written in the author's usual lucid and persuasive style. The author is exclusively committed to the Freudian psychoanalytic concepts and the conviction that advances in psychosomatic understanding will come only from greater knowledge of unconscious forces. He sees body and somatic structure as the recipient of a million years of evolutionary and survival experience. Much of what goes wrong somatically is seen as the punishment of the Ego and Super-Ego for the great instinctual urges that are unacceptable, often operating, as in tuberculosis, as a thinly disguised death wish. Thus the Unconscious determines alterations in function which ultimately become woven irreversibly into structure. After 45, he sees little opportunity for modification by analysis of the structural changes.

Structural-functional dynamic relationships are evidenced throughout the somatic make-up. The author presents cases of thyroid dysfunction, skin eruptions, Dupuytren's contracture, arthritis, bone tumor, etc., illustrating from the psychoanalytic concepts the type of interpretation possible from dream analysis, and its determining relationship to the somatic disturbance. The final chapter is an excellent resumé of the point of view, giving evidences of the growing contribution of the psychotherapist to the whole field of general medicine. Thus, while arthritis cannot be resolved purely to psychoanalytic principles, the joint of predilection may be so. The monograph is provocative of careful thought, opens up many avenues of theory and work for the psychoanalyst, is of genuine interest for all who are working, in whatever fashion, with the vast interrelated problems of medical disease and personality.

T. A. C. R.

Textbook of Nervous Diseases. By ROBERT BING. Translated and enlarged by Webb Haymaker from the 5th German edition. 838 pp. Illus. $10.00. *C. V. Mosby Company, St. Louis, 1939.*

Bing's textbook was one of the few really great textbooks of neurology and

deserved to rank with those of Gowers, Oppenheim and Dejerine. It was, of course, well known for its fine clinical descriptions and its lucid discussions of pathological anatomy. The book was first published in 1913 and unfortunately it eventually became out of date. It has, therefore, been revised by Webb Haymaker who has made many alterations and added much new material. The revision has been well done and valuable bibliographies are appended to each section. The elements which compose the present edition, therefore, are both excellent. It does not follow, however, that a mixture of excellent ingredients will always yield a happy result. Throughout the book there are evidences of two different and sometimes conflicting points of view. Nowhere is this more obvious than in the sections dealing with treatment where the most recent and, I hope, the more effective methods are presented beside the methods in vogue fifty years ago. According to Bing strychnine should be used in the treatment of tabes dorsalis, amyotonia congenita, poliomyelitis and polyneuritis and arsenic is recommended for a large percentage of all diseases of the nervous system. It is only fair to say, however, that Haymaker also describes the treatment in use today. The classification is quite confusing and illogical in my opinion. It is difficult to understand what considerations could justify the grouping together of myatonia congenita, occupational spasms, dystrophia myotonica, myasthenia gravis, periodic paralysis and kubisagari, for a more heterogenous list of diseases could scarcely be imagined. There are many fine things in this book and also many defects. It should not be recommended to anyone who is not sufficiently well informed to know what to accept and what to reject.

F. R. F.

BOOKS RECEIVED FOR REVIEW

The Medical Career and other papers. By HARVEY CUSHING. 302 pp. $2.50. Little, Brown and Company, Boston, 1940.

Unto the Fourth Generation. Gonorrhoea and Syphilis—What the layman should know. By IRVING SIMONS. 243 pp. Illus. $2.50. E. P. Dutton & Co., New York, 1940.

Virus and Rickettsial Diseases. A Symposium held at the Harvard School of Public Health, June 12–June 17, 1939. 907 pp. $6.50. Harvard University Press, Cambridge, Mass., 1940.

Injection Treatment. By PENN RIDDLE. 290 pp. Illus. $5.50. W. B. Saunders Company, Philadelphia, 1940.

Diseases of the Gallbladder and Bile Ducts. By WALTMAN WALTERS and ALBERT M. SNELL. 645 pp. Illus. $10.00. W. B. Saunders Company, Philadelphia, 1940.

Handbook of Orthopaedic Surgery. By ALFRED RIVES SHANDS. 2nd ed. 567 pp. Illus. $4.25. C. V. Mosby Company, St. Louis, 1940.

Pneumoconiosis (Silicosis): The Story of Dusty Lungs. By LEWIS GREGORY COLE and WILLIAM GREGORY COLE. 100 pp. Illus. $1.00. John B. Pierce Foundation, New York, 1940.

Manual of Cardiology. By WILLIAM D. REID. 364 pp. $3.50. Oxford University Press, London, New York, Toronto, 1940.

Preclinical Medicine. By MALFORD W. THEWLIS. 223 pp. $3.00. Williams & Wilkins Company, Baltimore, 1939.

Malarial Therapy of General Paralysis and Other Conditions. By WILLIAM H. KUPPER. 155 pp. Illus. $2.25. Edwards Brothers, Ann Arbor, Michigan, 1939.

Heil Hunger! Health under Hitler. By MARTIN GUMPERT. Translated from the German by Maurice Samuel. 128 pp. $1.75. Alliance Book Corporation: Longmans, Green & Company, New York and Toronto, 1940.

Experimental Investigations in Serum Allergy with reference to the Etiology of Rheumatic Joint Diseases. By EGON BRUUN. 229 pp. Illus. Danish Kr. 24. Einar Munksgaard, Copenhagen, and Humphrey Milford, London, 1940.

Diathermie Chirurgicale. By C. A. ARRAUD. 216 pp. Illus. 50 fr. Gauthier-Villars, Paris, 1939. (Les Actualités Physiothérapiques. I. Electrothérapie. 7.)

The Electrocardiogram in Congenital Cardiac Disease. By MAURICE A. SCHNITKER. 147 pp. Illus. $3.00. Harvard University Press, Cambridge, Mass., 1940.

Management of Obstetric Difficulties. By PAUL TITUS. 2nd ed. 968 pp. Illus. $10.00. C. V. Mosby Company, St. Louis, 1940.

A Manual for Diabetic Patients. By W. D. SANSUM, ALFRED E. KOEHLER, and RUTH BOWDEN. 227 pp. Illus. $3.25. *The Macmillan Company, New York, 1939.*

The book is divided into two parts—the first deals briefly with the history and pathological physiology of diabetes mellitus and outlines the aims and methods in therapy. Despite the fact that there is a glossary which gives explanations of the terms used, the language is quite technical and seems more adapted to the physician than to the layman. The reviewer asked a highly intelligent diabetic patient to read the book, and the foregoing opinion was shared by the patient. The chapter on insulin regulation oversimplifies the problem of adjustment, for the general rules laid down will hardly work in all cases.

Part two deals with dietary management and is extremely well done. Especially helpful are the sections devoted to menu planning, recipes, and substitution values.

There are not a few statements in the book with which the reviewer cannot entirely agree. But this is to be expected, since there are still so many controversial points and factors as yet unclarified in the disease diabetes mellitus.

J. E. H.

INDEX TO VOLUME LXVI

Albright, Fuller, Wm. V. Consolazio, Frederick S. Coombs, Hirsh W. Sulkowitch and John H. Talbott: Metabolic studies and therapy in a case of nephrocalcinosis with rickets and dwarfism 7
Alpert, Louis K., and Caroline B. Thomas: Renal function in hypertensive dogs. (Proceedings) 407
Arthus phenomenon, Studies on the site of sensitivity in the 106
Asthma, bronchial, The medical and surgical treatment of severe 34
Austrian, R. (see Gemmill, C. L.) 167

Biskind, Gerson R. (see Carnes, Wm. H.) 297
Book Reviews 65, 134, 199, 257, 330, 409
Breast, Cancer of the. (Proceedings) 327
Brown, Thomas McP., and John C. Nunemaker: Rat bite fever with arthritis due to streptobacillus moniliformis. (Proceedings) 325
Brown, Webster H., and A. Louis Dippel: The uses and limitations of soft tissue roentgenography in placenta praevia and in certain other obstetrical conditions 90
Broyles, Edwin N.: Treatment of laryngeal papilloma in children with estrogenic hormone 319

Carnes, Wm. H., and Gerson R. Biskind: The effect of testerosterone propionate on the development of tuberculosis in the immature male guinea pig 297
Cheyne-Stokes respiration, Cardiac arrhythmia during 335
Christian, Henry A.: Harvey Cushing Memorial Notice 1
Congenital malformation of the heart of the Eisenmenger type, Severe aortic insufficiency in association with a 156
Consolazio, Wm. F. (see Albright, Fuller) 7
Coombs, Frederick A. (see Albright, Fuller) 7
Cushing, Harvey, Memorial notice 1

Dieuaide, Francis R.: Hypertensive disease in the Chinese. (Proceedings) 408
Dippel, A. Louis (see Brown, Webster H.) 90

Enzymes, Chemistry of the 207

Finney, George G. (see Fisher, A. Murray) 263
Fisher, A. Murray, and George G. Finney: Lung abscess. Analysis of 88 cases 263
Florman, Alfred L., and Joseph Hafkenschiel: The eastern variety of Rocky Mountain spotted fever 123
Follis, Richard H., Jr. (see Rich, Arnold R.) 106
Follis, Richard H., Jr.: Studies on the cellular response in the early stages of the tuberculin reaction 245

Gemmill, Chalmers L.: The effect of stimulation on the fat and carbohydrate content of the gastrocnemius muscle in the phlorizinized rat 71
Gemmill, Chalmers L.: The effect of insulin on the glycogen content of isolated muscles 232
Gemmill, C. L., G. O. Gey and R. Austrian: The metabolism of tissue cultures of Walker rat sacroma 319 167
Gey, G. O. (see Gemmill, C. L.) 167
Goldenberg, Marcel (see Taussig, Helen B.) 323

Hafkenschiel, Joseph (see Florman, Alfred L.) 123
Hamilton, John D. (see Rich, Arnold R.) 185
Hamilton, John D. (see Rich, Arnold R.) 324
Harvey, A. M.: The action of quinine methochloride on neuromuscular transmission 52
Haviland, James W., and Perrin H. Long: Skin, conjunctival and scleral reactions in the course of treatment with sulfathiazole 313
Hellman, L. M., Wm. T. Moore and Landrum B. Shettles: Factors influencing plasmaprothrombin in the newborn infant. III. A study of the vitamin K activity of various naphthohydroquinone derivatives 379
Hesser, Frederick H.: Hypertrophia musculorum vera (dystrophia musculorum hyperplastica) associated with hypothyroidism: A case study 353
Hill, Justina H.: Experimental studies of sulfathiazole as a urinary antiseptic. (Proceedings) 404
Howell, William H.: The nature of the thromboplastic substance in tissues. (Proceedings) 406
Hypertensive disease in the Chinese. (Proceedings) 408
Hypertensive dogs, Renal function in. (Proceedings) 407
Hypertrophia musculorum vera (dystrophia musculorum hyperplastica) associated with hypothyroidism: A case study 353

Insulin, The effect of, on the glycogen content of isolated muscles 232

Johns Hopkins Medical Society, Proceedings 323, 404

Keeney, Edmund L.: The medical and surgical treatment of severe bronchial asthma 34

Laryngeal papilloma in children, Treatment of, with estrogenic hormone 319
Lewis, Warren H.: On the chromosomal nature of nucleoli 60
Liver, The experimental production of cirrhosis of the, by means of a deficient diet 185
Liver, The experimental production of cirrhosis of the, by means of a deficient diet. (Proceedings) 324
Long, Perrin H. (see Haviland, James W.) 313
Lung abscess. Analysis of 88 cases 263

Matthews, Edward, and W. Barry Wood: Cardiac arrhythmia during Cheyne-Stokes respiration 335

Moore, Wm. T. (see Hellman, L. M.) 379
Muscle, gastrocnemius, in the phlorizinized rat, The effect of stimulation on the fat and carbohydrate content of the 71

Nephrocalcinosis with rickets and dwarfism, Metabolic studies and therapy in a case of 7
Northrop, John H.: Chemistry of the Enzymes 207
Nucleoli, On the chromosomal nature of 60
Nunemaker, John C. (see Brown, Thomas McP.) 325

Patient as a Person 390
Pituitary gland, The native hormones of the posterior: The pressor and oxytocic principles 398
Placenta praevia and certain other obstetrical conditions, The uses and limitations of soft tissue roentgenography in 90
Prothrombin, plasma, in the newborn infant, Factors influencing. III. A study of the vitamin K activity of various naphthohydroquinone derivatives 379
Pseudo-thrombotic occlusion of the common femoral artery by cystic distension of the arterial wall 253

Quinine methochloride, The action of, on neuromuscular transmission 52

Rat bite fever with arthritis due to streptobacillus moniliformis. (Proceedings) 325
Rheumatic heart disease, Studies of cardiac enlargement in children with. (Proceedings) 323
Rich, Arnold R., and Richard H. Follis, Jr.: Studies on the site of sensitivity in the Arthus phenomenon 106
Rich, Arnold R., and John D. Hamilton: The experimental production of cirrhosis of the liver by means of a deficient diet 185
Rich, Arnold R., and John D. Hamilton: The experimental production of cirrhosis of the liver by means of a deficient diet. (Proceedings) 324
Robinson, G. Canby: The patient as a person 390
Rocky Mountain spotted fever, The eastern variety of 123
Rosenfeld, Morris: The native hormones of the posterior pituitary gland: The pressor and oxytocic principles 398

Sarcoma 319, The metabolism of tissue cultures of Walker rat 167
Semans, James H. (see Taussig, Helen B.) 156
Shettles, Landrum B. (see Hellman, L. M.) 379
Speert, Harold: The placental transmission of sulfanilamide and its effects upon the the fetus and newborn 139
Stafford, Edward S.: Pseudo-thrombotic occlusion of the common femoral artery by cystic distension of the arterial wall 253
Sulfanilamide, The placental transmission of, and its effects upon the fetus and newborn 139
Sulfathiazole as a urinary antiseptic, Experimental studies of. (Proceedings) 404
Sulfathiazole, Skin, conjunctival and scleral reactions in the course of treatment with 313
Sulkowitch, Hirsh W. (see Albright, Fuller) 7

Talbott, John H. (see Albright, Fuller) 7
Taussig, Helen B., and James H. Semans: Severe aortic insufficiency in association with a congenital malformation of the heart of the Eisenmenger type 156
Taussig, Helen B., and Marcel Goldenberg: Studies of cardiac enlargement in children with rheumatic heart disease. (Proceedings) 323
Thomas, Caroline B. (see Alpert, Louis K.) 407
Thromboplastic substance in tissues, The nature of the. (Proceedings) 406
Trimble, I. Ridgeway: Cancer of the breast. (Proceedings) 327
Tuberculin reaction, Studies on the cellular response in the early stages of the 245
Tuberculosis in the immature male guinea pig, The effect of testerosterone propionate on the development of 297

Wood, W. Barry (see Matthews, Edward) 335

Medical Library

VOLUME LXVI NUMBER 6

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Cardiac Arrhythmia during Cheyne-Stokes Respiration. Edward Matthews and W. Barry Wood, Jr. 335

Hypertrophia Musculorum Vera (Dystrophia Musculorum Hyperplastica) Associated with Hypothyroidism: A Case Study. Frederick H. Hesser 353

Factors Influencing Plasma Prothrombin in the Newborn Infant. III. A Study of the Vitamin K Activity of Various Naphthohydroquinone Derivatives. L. M. Hellman, William T. Moore, and Landrum B. Shettles 379

The Patient as a Person. G. Canby Robinson, M.D. 390

The Native Hormones of the Posterior Pituitary Gland: The Pressor and Oxytocic Principles. Morris Rosenfeld ... 398

Proceedings of a Meeting of the Johns Hopkins Medical Society. 404

Book Reviews 409

Index to Volume LXVI 416

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These vitamin mixtures may be a commercial success, but they cannot replace the need for adequate amounts of a well balanced diet. Vitamin mixtures may have some reason behind the selection of vitamins which they contain and the amounts of each that are present, but if so, the reason is a "trade secret" which has not been divulged. Certainly there is no rational basis for placing in capsules amounts of vitamins A and D which, in the amounts recommended by the firms, are several times a therapeutic dose, and amounts of vitamin B_1 and riboflavin which are almost insignificant compared to the contribution which an adequate diet would supply. Advertising campaigns and other efforts to promote the indiscriminate use of expensive polyvitamin mixtures are unfortunate because the "educational" program is not based on sound principles of nutrition or medicine. It is not denied that there may be a limited field in which polyvitamin mixtures might be useful, if the qualitative and quantitative composition of the mixtures were scientifically rational. It is conceivable, for example, that a physician might desire to provide a patient on a restricted therapeutic diet with vitamins which ordinarily are supplied by an adequate diet. But the available preparations of vitamin mixtures obviously cannot solve the problem of giving a patient the vitamins and food which he needs. The use of vitamin mixtures by the public is no assurance of "good health"; their daily use is no guaranty that all of the vitamins which a person must secure will be provided.

The Committee reaffirmed the policy of the Council on Pharmacy and Chemistry not to accept irrational fabricated mixtures of vitamins. The two councils concurred.

Report of the Council on Pharmacy and Chemistry and the Council on Foods: The status of certain questions concerning vitamins, based on recommendations of the Cooperative Committee on Vitamins, J. A. M. A., 113:595, August 12, 1939

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